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(54) Title: NANOPARTICLES HAVING OLIGONUCLEOTIDES ATTACHED THERETO AND USES THEREFOR

(57) Abstract

The invention provides methods of detecting a nucleic acid. The methods comprise contacting the nucleic acid with one or more types of particles having oligonucleotides attached thereto. In one embodiment of the method, the oligonucleotides are attached to nanoparticles and have sequences complementary to portions of the sequence of the nucleic acid. A detectable change (preferably a color change) is brought about as a result of the hybridization of the oligonucleotides on the nanoparticles to the nucleic acid. The invention also provides compositions and kits comprising particles. The invention further provides nanomaterials and nanostructures comprising nanoparticles and methods of nanofabrication utilizing the nanoparticles. Finally, the invention provides a method of separating a selected nucleic acid from other nucleic acids.

NANOPARTICLES HAVING OLIGONUCLEOTIDES ATTACHED THERETO AND USES THEREFOR

Benefit of provisional application number
60/031,809, filed July 29, 1996, is hereby claimed.

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FIELD OF THE INVENTION

The invention relates to methods of detecting
nucleic acids, whether natural or synthetic, and whether
modified or unmodified. This invention also relates to

15   methods of nanofabrication. Finally, the invention
relates to methods of separating a selected nucleic acid
from other nucleic acids.

BACKGROUND OF THE INVENTION

20   The development of methods for detecting and
sequencing nucleic acids is critical to the diagnosis of
genetic, bacterial, and viral diseases. See Mansfield,
E.S. et al. *Molecular and Cellular Probes*, 9, 145-156
(1995). At present, there are a variety of methods used

25   for detecting specific nucleic acid sequences. Id.
However, these methods are complicated, time-consuming
and/or require the use of specialized and expensive
equipment. A simple, fast method of detecting nucleic
acids which does not require the use of such equipment

30   would clearly be desirable.

A variety of methods have been developed for
assembling metal and semiconductor colloids into
nanomaterials. These methods have focused on the use of
covalent linker molecules that possess functionalities at

35   opposing ends with chemical affinities for the colloids
of interest. One of the most successful approaches to

2

date, Brust et al., *Adv. Mater.*, 7, 795-797 (1995), involves the use of gold colloids and well-established thiol adsorption chemistry, Bain & Whitesides, *Angew. Chem. Int. Ed. Engl.*, 28, 506-512 (1989) and Dubois &

5   Nuzzo, *Annu. Rev. Phys. Chem.*, 43, 437-464 (1992). In this approach, linear alkanedithiols are used as the particle linker molecules. The thiol groups at each end of the linker molecule covalently attach themselves to the colloidal particles to form aggregate structures.

10 The drawbacks of this method are that the process is difficult to control and the assemblies are formed irreversibly. Methods for systematically controlling the assembly process are needed if the materials properties of these structures are to be exploited fully.

15   The potential utility of DNA for the preparation of biomaterials and in nanofabrication methods has been recognized. In this work, researchers have focused on using the sequence-specific molecular recognition properties of oligonucleotides to design impressive

20 structures with well-defined geometric shapes and sizes. Shekhtman et al., *New J. Chem.*, 17, 757-763 (1993); Shaw & Wang, *Science*, 260, 533-536 (1993); Chen et al., *J. Am Chem. Soc.*, 111, 6402-6407 (1989); Chen & Seeman, *Nature*, 350, 631-633 (1991); Smith and Feigon, *Nature*, 356, 164-

25 168 (1992); Wang et al., *Biochem.*, 32, 1899-1904 (1993); Chen et al,, *Biochem.*, 33, 13540-13546 (1994); Marsh et al., *Nucleic Acids Res.*, 23, 696-700 (1995); Mirkin, *Annu. Review Biophys. Biomol. Struct.*, 23, 541-576 (1994); Wells, *J. Biol. Chem.*, 263, 1095-1098 (1988);

30 Wang et al., *Biochem.*, 30, 5667-5674 (1991). However, the theory of producing DNA structures is well ahead of experimental confirmation. Seeman et al., *New J. Chem.*, 17, 739-755 (1993).

SUMMARY OF THE INVENTION

The invention provides methods of detecting nucleic acids. In one embodiment, the method comprises contacting a nucleic acid with one or more types of
5   nanoparticles having oligonucleotides attached thereto (nanoparticle-oligonucleotide conjugates). The nucleic acid has at least two portions, and the oligonucleotides on each of the types of nanoparticles have a sequence complementary to the sequence of one of the portions of
10   the nucleic acid. The contacting takes place under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with the nucleic acid. The hybridization of the oligonucleotides on the nanoparticles with the nucleic acid results in a
15   detectable change.

In another embodiment, the method comprises contacting a nucleic acid with at least two types of nanoparticles having oligonucleotides attached thereto. The oligonucleotides on the first type of nanoparticles
20   have a sequence complementary to a first portion of the sequence of the nucleic acid. The oligonucleotides on the second type of nanoparticles have a sequence complementary to a second portion of the sequence of the nucleic acid. The contacting takes place under
25   conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with the nucleic acid, and a detectable change brought about by this hybridization is observed.

In a further embodiment, the method comprises
30   providing a substrate having a first type of nanoparticles attached thereto. The first type of nanoparticles has oligonucleotides attached thereto, and the oligonucleotides have a sequence complementary to a first portion of the sequence of a nucleic acid. The
35   substrate is contacted with the nucleic acid under

conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with the nucleic acid. Then, a second type of nanoparticles having oligonucleotides attached thereto is provided. The

5 oligonucleotides have a sequence complementary to one or more other portions of the sequence of the nucleic acid, and the nucleic acid bound to the substrate is contacted with the second type of nanoparticle-oligonucleotide conjugates under conditions effective to allow

10 hybridization of the oligonucleotides on the second type of nanoparticles with the nucleic acid. A detectable change may be observable at this point. The method may further comprise providing a binding oligonucleotide having a selected sequence having at least two portions,

15 the first portion being complementary to at least a portion of the sequence of the oligonucleotides on the second type of nanoparticles. The binding oligonucleotide is contacted with the second type of nanoparticle-oligonucleotide conjugates bound to the

20 substrate under conditions effective to allow hybridization of the binding oligonucleotide to the oligonucleotides on the nanoparticles. Then, a third type of nanoparticles having oligonucleotides attached thereto, the oligonucleotides having a sequence

25 complementary to the sequence of a second portion of the binding oligonucleotide, is contacted with the binding oligonucleotide bound to the substrate under conditions effective to allow hybridization of the binding oligonucleotide to the oligonucleotides on the

30 nanoparticles. Finally, the detectable change produced by these hybridizations is observed.

In yet another embodiment, the method comprises contacting a nucleic acid with a substrate having oligonucleotides attached thereto, the oligonucleotides

35 having a sequence complementary to a first portion of the

sequence of the nucleic acid. The contacting takes place under conditions effective to allow hybridization of the oligonucleotides on the substrate with the nucleic acid. Then, the nucleic acid bound to the substrate is

5   contacted with a first type of nanoparticles having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to a second portion of the sequence of the nucleic acid. The contacting takes place under conditions effective to allow hybridization

10 of the oligonucleotides on the nanoparticles with the nucleic acid. Next, the first type of nanoparticle-oligonucleotide conjugates bound to the substrate is contacted with a second type of nanoparticles having oligonucleotides attached thereto, the oligonucleotides

15 on the second type of nanoparticles having a sequence complementary to at least a portion of the sequence of the oligonucleotides on the first type of nanoparticles, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the first

20 and second types of nanoparticles. Finally, a detectable change produced by these hybridizations is observed.

In another embodiment, the method comprises contacting a nucleic acid with a substrate having oligonucleotides attached thereto, the oligonucleotides

25 having a sequence complementary to a first portion of the sequence of the nucleic acid. The contacting takes place under conditions effective to allow hybridization of the oligonucleotides on the substrate with the nucleic acid. Then, the nucleic acid bound to the substrate is

30 contacted with liposomes having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to a portion of the sequence of the nucleic acid. This contacting takes place under conditions effective to allow hybridization of the oligonucleotides

35 on the liposomes with the nucleic acid. Next, the

liposome-oligonucleotide conjugates bound to the substrate are contacted with a first type of nanoparticles having at least a first type oligonucleotides attached thereto. The first type of

5 oligonucleotides have a hydrophobic group attached to the end not attached to the nanoparticles, and the contacting takes place under conditions effective to allow attachment of the oligonucleotides on the nanoparticles to the liposomes as a result of hydrophobic interactions.

10 A detectable change may be observable at this point. The method may further comprise contacting the first type of nanoparticle-oligonucleotide conjugates bound to the liposomes with a second type of nanoparticles having oligonucleotides attached thereto. The first type of

15 nanoparticles have a second type of oligonucleotides attached thereto which have a sequence complementary to at least a portion of the sequence of the oligonucleotides on the second type of nanoparticles, and the oligonucleotides on the second type of nanoparticles

20 having a sequence complementary to at least a portion of the sequence of the second type of oligonucleotides on the first type of nanoparticles. The contacting takes place under conditions effective to allow hybridization of the oligonucleotides on the first and second types of

25 nanoparticles. Then, a detectable change is observed.

In yet another embodiment, the method comprises providing nanoparticles having oligonucleotides attached thereto and providing one or more types of binding oligonucleotides. Each of the binding oligonucleotides

30 has two portions. The sequence of one portion is complementary to the sequence of one of the portions of the nucleic acid, and the sequence of the other portion is complementary to the sequence of the oligonucleotides on the nanoparticles. The nanoparticle-oligonucleotide

35 conjugates and the binding oligonucleotides are contacted

7

under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with the binding oligonucleotides. The nucleic acid and the binding oligonucleotides are contacted under conditions effective
5   to allow hybridization of the binding oligonucleotides with the nucleic acid. Then, a detectable change is observed. The nanoparticle-oligonucleotide conjugates may be contacted with the binding oligonucleotides prior to being contacted with the nucleic acid, or all three
10   may be contacted simultaneously.

In another embodiment, the method comprises contacting a nucleic acid with at least two types of particles having oligonucleotides attached thereto. The oligonucleotides on the first type of particles have a
15   sequence complementary to a first portion of the sequence of the nucleic acid and have energy donor molecules on the ends not attached to the particles. The oligonucleotides on the second type of particles have a sequence complementary to a second portion of the
20   sequence of the nucleic acid and have energy acceptor molecules on the ends not attached to the particles. The contacting takes place under conditions effective to allow hybridization of the oligonucleotides on the particles with the nucleic acid, and a detectable change
25   brought about by this hybridization is observed. The energy donor and acceptor molecules may be fluorescent molecules.

The invention further provides kits for detecting nucleic acids. In one embodiment, the kit comprises at
30   least one container, the container holding at least two types of nanoparticles having oligonucleotides attached thereto. The oligonucleotides on the first type of nanoparticles have a sequence complementary to the sequence of a first portion of a nucleic acid. The
35   oligonucleotides on the second type of nanoparticles have

a sequence complementary to the sequence of a second
portion of the nucleic acid.

Alternatively, the kit may comprise at least two
containers. The first container holds nanoparticles
5   having oligonucleotides attached thereto which have a
sequence complementary to the sequence of a first portion
of a nucleic acid. The second container holds
nanoparticles having oligonucleotides attached thereto
which have a sequence complementary to the sequence of a
10   second portion of the nucleic acid.

In a further embodiment, the kit comprises at least
one container. The container holds metallic or
semiconductor nanoparticles having oligonucleotides
attached thereto. The oligonucleotides have a sequence
15   complementary to portion of a nucleic acid and have
fluorescent moelcules attached to the ends of the
oligonucleotides not attached to the nanoparticles.

In yet another embodiment, the kit comprises a
substrate, the substrate having attached thereto
20   nanoparticles, the nanoparticles having oligonucleotides
attached thereto which have a sequence complementary to
the sequence of a first portion of a nucleic acid. The
kit also includes a first container holding nanoparticles
having oligonucleotides attached thereto which have a
25   sequence complementary to the sequence of a second
portion of the nucleic acid. The kit further includes a
second container holding a binding oligonucleotide having
a selected sequence having at least two portions, the
first portion being complementary to at least a portion
30   of the sequence of the oligonucleotides on the
nanoparticles in the first container. The kit also
includes a third container holding nanoparticles having
oligonucleotides attached thereto, the oligonucleotides
having a sequence complementary to the sequence of a
35   second portion of the binding oligonucleotide.

In another embodiment, the kit comprises a substrate having oligonucleotides attached thereto which have a sequence complementary to the sequence of a first portion of a nucleic acid, a first container holding

5   nanoparticles having oligonucleotides attached thereto which have a sequence complementary to the sequence of a second portion of the nucleic acid, and a second container holding nanoparticles having oligonucleotides attached thereto which have a sequence complementary to

10   at least a portion of the oligonucleotides attached to the nanoparticles in the first container.

In yet another embodiment, the kit comprises a substrate, a first container holding nanoparticles, a second container holding a first type of oligonucleotides

15   having a sequence complementary to the sequence of a first portion of a nucleic acid, a third container holding a second type of oligonucleotides having a sequence complementary to the sequence of a second portion of the nucleic acid, and a fourth container

20   holding a third type of oligonucleotides having a sequence complementary to at least a portion of the sequence of the second type of oligonucleotides.

In a further embodiment, the kit comprises a substrate having oligonucleotides attached thereto which

25   have a sequence complementary to the sequence of a first portion of a nucleic acid. The kit also includes a first container holding liposomes having oligonucleotides attached thereto which have a sequence complementary to the sequence of a second portion of the nucleic acid and

30   a second container holding nanoparticles having at least a first type of oligonucleotides attached thereto, the first type of oligonucleotides having a hydrophobic group attached to the end not attached to the nanoparticles so that the nanoparticles can be attached to the liposomes

35   by hydrophobic interactions. The kit may further

comprise a third container holding a second type of nanoparticles having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to at least a portion of the sequence of a second type of
5   oligonucleotides attached to the first type of nanoparticles. The second type of oligonucleotides attached to the first type of nanoparticles have a sequence complementary to the sequence of the oligonucleotides on the second type of nanoparticles.

10   In a further embodiment, the kit comprises a first container holding nanoparticles having oligonucleotides attached thereto. The kit also includes one or more additional containers, each container holding a binding oligonucleotide. Each binding oligonucleotide has a
15   first portion which has a sequence complementary to at least a portion of the sequence of oligonucleotides on the nanoparticles and a second portion which has a sequence complementary to the sequence of a portion of a nucleic acid to be detected. The sequences of the second
20   portions of the binding oligonucleotides may be different as long as each sequence is complementary to a portion of the sequence of the nucleic acid to be detected.

In yet another embodiment, the kit comprises a container holding one type of nanoparticles having
25   oligonucleotides attached thereto and one or more types of binding oligonucleotides. Each of the types of binding oligonucleotides has a sequence comprising at least two portions. The first portion is complementary to the sequence of the oligonucleotides on the
30   nanoparticles, whereby the binding oligonucleotides are hybridized to the oligonucleotides on the nanoparticles in the container(s). The second portion is complementary to the sequence of a portion of the nucleic acid.

In another alternative embodiment, the kit comprises
35   at least three containers. The first container holds

nanoparticles. The second container holds a first
oligonucleotide having a sequence complementary to the
sequence of a first portion of a nucleic acid. The third
container holds a second oligonucleotide having a

5   sequence complementary to the sequence of a second
portion of the nucleic acid. The kit may further
comprise a fourth container holding a binding
oligonucleotide having a selected sequence having at
least two portions, the first portion being complementary

10   to at least a portion of the sequence of the second
oligonucleotide, and a fifth container holding an
oligonucleotide having a sequence complementary to the
sequence of a second portion of the binding
oligonucleotide.

15   In another embodiment, the kit comprises one or two
containers, the container(s) holding two types of
particles. The first type of particles having
oligonucleotides attached thereto that have a sequence
complementary to a first portion of the sequence of a

20   nucleic acid and have energy donor molecules attached to
the ends not attached to the nanoparticles. The second
type of particles having oligonucleotides attached
thereto that have a sequence complementary to a second
portion of the sequence of a nucleic acid and have energy

25   acceptor molecules attached to the ends not attached to
the nanoparticles. The energy donors and acceptors may
be fluorescent molecules.

       The invention also provides a substrate having
nanoparticles attached thereto. The nanoparticles may

30   have oligonucleotides attached thereto which have a
sequence complementary to the sequence of a first portion
of a nucleic acid.

       The invention further provides a method of
nanofabrication. The method comprises providing at least

35   one type of linking oligonucleotide having a selected

sequence, the sequence of each type of linking
oligonucleotide having at least two portions. The method
further comprises providing one or more types of
nanoparticles having oligonucleotides attached thereto,

5   the oligonucleotides on each type of nanoparticles having
a sequence complementary to a portion of the sequence of
a linking oligonucleotide. The linking oligonucleotides
and nanoparticles are contacted under conditions
effective to allow hybridization of the oligonucleotides

10 on the nanoparticles to the linking oligonucleotides so
that a desired nanomaterial or nanostructure is formed.

The invention provides another method of
nanofabrication. This method comprises providing at
least two types of nanoparticles having oligonucleotides

15 attached thereto. The oligonucleotides on the first type
of nanoparticles have a sequence complementary to that of
the oligonucleotides on the second type of nanoparticles.
The oligonucleotides on the second type of nanoparticles
have a sequence complementary to that of the

20 oligonucleotides on the first type of nanoparticle-
oligonucleotide conjugates. The first and second types
of nanoparticles are contacted under conditions effective
to allow hybridization of the oligonucleotides on the
nanoparticles to each other so that a desired

25 nanomaterial or nanostructure is formed.

The invention further provides nanomaterials or
nanostructures composed of nanoparticles having
oligonucleotides attached thereto, the nanoparticles
being held together by oligonucleotide connectors.

30 The invention also provides a composition comprising
at least two types of nanoparticles having
oligonucleotides attached thereto. The oligonucleotides
on the first type of nanoparticles have a sequence
complementary to the sequence of a first portion of a

35 nucleic acid or a linking oligonucleotide. The

oligonucleotides on the second type of nanoparticles have
a sequence complementary to the sequence of a second
portion of the nucleic acid or linking oligonucleotide.

The invention further provides an assembly of
5   containers comprising a first container holding
nanoparticles having oligonucleotides attached thereto,
and a second container holding nanoparticles having
oligonucleotides attached thereto. The oligonucleotides
attached to the nanoparticles in the first container have
10   a sequence complementary to that of the oligonucleotides
attached to the nanoparticles in the second container.
The oligonucleotides attached to the nanoparticles in the
second container have a sequence complementary to that of
the oligonucleotides attached to the nanoparticles in the
15   first container.

The invention also provides a nanoparticle having a
plurality of different oligonucleotides attached to it.

Finally, the invention provides a method of
separating a selected nucleic acid having at least two
20   portions from other nucleic acids. The method comprises
providing one or more types of nanoparticles having
oligonucleotides attached thereto, the oligonucleotides
on each of the types of nanoparticles having a sequence
complementary to the sequence of one of the portions of
25   the selected nucleic acid. The selected and other
nucleic acids are contacted with the nanoparticles under
conditions effective to allow hybridization of the
oligonucleotides on the nanoparticles with the selected
nucleic acid so that the nanoparticles hybridized to the
30   selected nucleic acid aggregate and precipitate.

As used herein, a "type of oligonucleotides" refers
to a plurality of oligonucleotide molecules having the
same sequence. A "type of nanoparticles having
oligonucleotides attached thereto" refers to a plurality
35   of nanoparticles having the same type(s) of

14

oligonucleotides attached to them. "Nanoparticles having
oligonucleotides attached thereto" are also sometimes
referred to as "nanoparticle-oligonucleotide conjugates"
or, in the case of the detection methods of the
5   invention, "nanoparticle-oligonucleotide probes,"
"nanoparticle probes," or just "probes."

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Schematic diagram illustrating the
10   formation of nanoparticle aggregates by combining
nanoparticles having complementary oligonucleotides
attached to them, the nanoparticles being held together
in the aggregates as a result of the hybridization of the
complementary oligonucleotides. X represents any
15   covalent anchor (such as $-S(CH_2)_3OP(O)(O^-)-$, where S is
joined to a gold nanoparticle). For the sake of
simplicity in Figure 1 and some subsequent figures, only
one oligonucleotide is shown to be attached to each
particle but, in fact, each particle has several
20   oligonucleotides attached to it. Also, it is important
to note that in Figure 1 and subsequent figures, the
relative sizes of the gold nanoparticles and the
oligonucleotides are not drawn to scale.

Figure 2: Schematic diagram illustrating a system
25   for detecting nucleic acid using nanoparticles having
oligonucleotides attached thereto. The oligonucleotides
on the two nanoparticles have sequences complementary to
two different portions of the single-stranded DNA shown.
As a consequence, they hybridize to the DNA producing
30   detectable changes (forming aggregates and producing a
color change).

Figure 3: Schematic diagram of a variation of the
system shown in Figure 2. The oligonucleotides on the
two nanoparticles have sequences complementary to two
35   different portions of the single-stranded DNA shown which

are separated by a third portion which is not complementary to the oligonucleotides on the nanoparticles. Also shown is an optional filler oligonucleotide which can be used to hybridize with the

5   noncomplementary portion of the single-stranded DNA. When the DNA, nanoparticles and filler oligonucleotides are combined, the nanoparticles aggregate, with the formation of nicked, double-stranded oligonucleotide connectors.

10   Figure 4: Schematic diagram illustrating reversible aggregation of nanoparticles having oligonucleotides attached thereto as a result of hybridization and de-hybridization with a linking oligonucleotide. The illustrated linking oligonucleotide is a double-stranded

15   DNA having overhanging termini (sticky ends) which are complementary to the oligonucleotides attached to the nanoparticles.

          Figure 5: Schematic diagram illustrating the formation of nanoparticle aggregates by combining

20   nanoparticles having oligonucleotides attached thereto with linking oligonucleotides having sequences complementary to the oligonucleotides attached to the nanoparticles.

          Figure 6: Cuvettes containing two types of gold

25   colloids, each having a different oligonucleotide attached thereto and a linking double-stranded oligonucleotide with sticky ends complementary to the oligonucleotides attached to the nanoparticles (see Figure 4). Cuvette A - at 80°C, which is above the Tm of

30   the linking DNA; de-hybridized (thermally denatured). The color is dark red. Cuvette B - after cooling to room temperature, which is below the Tm of the linking DNA; hybridization has taken place, and the nanoparticles have aggregated, but the aggregates have not precipitated.

35   The color is purple. Cuvette C - after several hours at

room temperature, the aggregated nanoparticles have settled to the bottom of the cuvette. The solution is clear, and the precipitate is pinkish gray. Heating B or C will result in A.

5   Figure 7: A graph of absorbance versus wavelength in nm showing changes in absorbance when gold nanoparticles having oligonucleotides attached thereto aggregate due to hybridization with linking oligonucleotides upon lowering of the temperature, as
10   illustrated in Figure 4.

       Figures 8A-B: Figure 8A is a graph of change in absorbance versus temperature/time for the system illustrated in Figure 4. At low temperatures, gold nanoparticles having oligonucleotides attached thereto
15   aggregate due to hybridization with linking oligonucleotides (see Figure 4). At high temperature (80ºC), the nanoparticles are de-hybridized. Changing the temperature over time shows that this is a reversible process. Figure 8B is a graph of change in absorbance
20   versus temperature/time performed in the same manner using an aqueous solution of unmodified gold nanoparticles. The reversible changes seen in Figure 8A are not observed.

       Figures 9A-B: Transmission Electron Microscope
25   (TEM) images. Figure 9A is a TEM image of aggregated gold nanoparticles held together by hybridization of the oligonucleotides on the gold nanoparticles with linking oligonucleotides. Figure 9B is a TEM image of a two-dimensional aggregate showing the ordering of the linked
30   nanoparticles.

       Figure 10: Schematic diagram illustrating the formation of thermally-stable triple-stranded oligonucleotide connectors between nanoparticles having the pyrimidine:purine:pyrimidine motif. Such triple-
35   stranded connectors are stiffer than double-stranded

connectors. In Figure 10, one nanoparticle has an oligonucleotide attached to it which is composed of all purines, and the other nanoparticle has an oligonucleotide attached to it which is composed of all

5 pyrimidines. The third oligonucleotide for forming the triple-stranded connector (not attached to a nanoparticle) is composed of pyrimidines.

Figure 11: Schematic diagram illustrating the formation of nanoparticle aggregates by combining

10 nanoparticles having complementary oligonucleotides attached to them, the nanoparticles being held together in the aggregates as a result of the hybridization of the complementary oligonucleotides. In Figure 11, the circles represent the nanoparticles, the formulas are

15 oligonucleotide sequences, s is the thio-alkyl linker, and the squiggles on the circles represent other oligonucleotide molecules of the same sequence as indicated that cover the surface of the nanoparticles. The multiple oligonucleotides on the two types of

20 nanoparticles can hybridize to each other, leading to the formation of an aggregate structure.

Figure 12: Schematic diagrams illustrating systems for detecting nucleic acid using nanoparticles having oligonucleotides attached thereto. Oligonucleotide-

25 nanoparticle conjugates 1 and 2 and single-stranded oligonucleotide targets 3, 4, 5, 6 and 7 are illustrated. In Figure 12, the circles represent the nanoparticles, the formulas are oligonucleotide sequences, and the dotted lines represent connecting links of nucleotide.

30 Figures 13A-B: Schematic diagrams illustrating systems for detecting DNA (analyte DNA) using nanoparticles and a transparent substrate.

Figures 14A-B: Figure 14A is a graph of absorbance versus wavelength in nm showing changes in absorbance

35 when gold nanoparticles having oligonucleotides attached

thereto (one population of which is in solution and one
population of which is attached to a transparent
substrate as illustrated in Figure 13B) aggregate due to
hybridization with linking oligonucleotides. Figure 14B
5   a graph of change in absorbance for the hybridized system
referred to in Figure 14A as the temperature is increased
(melted).

       Figure 15: Schematic diagrams illustrating systems
for detecting nucleic acid using nanoparticles having
10   oligonucleotides attached thereto. Oligonucleotide-
nanoparticle conjugates 1 and 2 and single-stranded
oligonucleotide targets 3, 4, 5, 6, 7 and 8 are
illustrated. In Figure 15, the circles represent the
nanoparticles, the formulas are oligonucleotide
15   sequences, and S represents the thio-alkyl linker.

       Figure 16: Schematic diagrams illustrating systems
for detecting nucleic acid using nanoparticles having
oligonucleotides attached thereto. Oligonucleotide-
nanoparticle conjugates 1 and 2, single-stranded
20   oligonucleotide targets of different lengths, and filler
oligonucleotides of different lengths are illustrated.
In Figure 16; the circles represent the nanoparticles,
the formulas are oligonucleotide sequences, and S
represents the thio-alkyl linker.

25   Figure 17: Schematic diagrams illustrating
nanoparticle-oligonucleotide conjugates and systems for
detecting nucleic acid using nanoparticles having
oligonucleotides attached thereto. In Figure 17, the
circles represent the nanoparticles, the straight lines
30   represent oligonucleotide chains (bases not shown), two
closely-spaced parallel lines represent duplex segments,
and the small letters indicate specific nucleotide
sequences (a is complementary to a', b is complementary
to b', etc.).

Figure 18: Schematic diagram illustrating a system for detecting nucleic acid using liposomes (large double circle), nanoparticles (small dark circles) and a transparent substrate. In figure 18, the filled-in

5   squares represent cholesteryl groups, the squiggles represent oligonucleotides, and the ladders represent double-stranded (hybridized) oligonucleotides.

Figures 19A-B: Figure 19A is a graph of absorbance versus wavelength in nm showing changes in absorbance

10   when gold nanoparticle-oligonucleotide conjugates assemble in multiple layers on a transparent substrate as illustrated in Figure 13A. Figure 19B is a graph of change in absorbance for the hybridized system referred to in Figure 19A as the temperature is increased

15   (melted).

Figures 20A-B: Illustrations of schemes using fluorescent-labeled oligonucleotides attached to metallic or semiconductor quenching nanoparticles (Figure 20A) or to non-metallic, non-semiconductor particles (Figure

20   20B).

DETAILED DESCRIPTION OF THE
PRESENTLY PREFERRED EMBODIMENTS

Nanoparticles useful in the practice of the

25   invention include metal (e.g., gold, silver, copper and platinum), semiconductor (e.g., CdSe and CdS) and magnetic (e.g., ferromagnetite) colloidal materials. Other nanoparticles useful in the practice of the invention include ZnS, ZnO, TiO_2, AgI, AgBr, HgI_2, PbS,

30   PbSe, ZnTe, CdTe, In_2S_3, In_2Se_3, Cd_3P_2, Cd_3As_2, InAs, and GaAs. The size of the nanoparticles is preferably from about 5 nm to about 150 nm (mean diameter), more preferably from about 5 to about 50 nm, most preferably from about 10 to about 30 nm.

20

Methods of making metal, semiconductor and magnetic nanoparticles are well-known in the art. See, *e.g.*, Schmid, G. (ed.) *Clusters and Colloids* (VCH, Weinheim, 1994); Hayat, M.A. (ed.) *Colloidal Gold: Principles,*

5 *Methods, and Applications* (Academic Press, San Diego, 1991); Massart, R., *IEEE Taransactions On Magnetics,* **17,** 1247 (1981); Ahmadi, T.S. et al., *Science,* **272,** 1924 (1996); Henglein, A. et al., *J. Phys. Chem.,* **99,** 14129 (1995); Curtis, A.C., et al., *Angew. Chem. Int. Ed.*

10 *Engl.,* **27,** 1530 (1988).

Methods of making ZnS, ZnO, TiO_2, AgI, AgBr, HgI_2, PbS, PbSe, ZnTe, CdTe, In_2S_3, In_2Se_3, Cd_3P_2, Cd_3As_2, InAs, and GaAs nanoparticles are also known in the art. See, *e.g.*, Weller, *Angew. Chem. Int. Ed. Engl.,* **32,** 41 (1993);

15 Henglein, *Top. Curr. Chem.,* **143,** 113 (1988); Henglein, *Chem. Rev.,* **89,** 1861 (1989); Brus, *Appl. Phys. A.,* **53,** 465 (1991); Bahncmann, in <u>Photochemical Conversion and Storage of Solar Energy</u> (eds. Pelizetti and Schiavello 1991), page 251; Wang and Herron, *J. Phys. Chem.,* **95,** 525

20 (1991); Olshavsky et al., *J. Am. Chem. Soc.,* **112,** 9438 (1990); Ushida et al., *J. Phys. Chem.,* **95,** 5382 (1992).

Suitable nanoparticles are also commercially available from, *e.g.*, Ted Pella, Inc. (gold), Amersham Corporatrion (gold) and Nanoprobes, Inc. (gold).

25 Presently preferred for use in detecting nucleic acids are gold nanoparticles. Gold colloidal particles have high extinction coefficients for the bands that give rise to their beautiful colors. These intense colors change with particle size, concentration, interparticle

30 distance, and extent of aggregation and shape (geometry) of the aggregates, making these materials particularly attractive for colorimetric assays. For instance, hybridization of oligonucleotides attached to gold nanoparticles with oligonucleotides and nucleic acids

results in an immediate color change visible to the naked eye (see, _e.g._, the Examples).

Gold nanoparticles are also presently preferred for use in nanofabrication for the same reasons given above and because of their stability, ease of imaging by electron microscopy, and well-characterized modification with thiol functionalities (see below).

The nanoparticles, the oligonucleotides or both are functionalized in order to attach the oligonucleotides to the nanoparticles. Such methods are known in the art. For instance, oligonucleotides functionalized with alkanethiols at their 3'-termini or 5'-termini readily attach to gold nanoparticles. See Whitesides, _Proceedings of the Robert A. Welch Foundation 39th Conference On Chemical Research Nanophase Chemistry_, Houston, TX, pages 109-121 (1995). See also, Mucic et al. _Chem. Commun._ 555-557 (1996) (describes a method of attaching 3' thiol DNA to flat gold surfaces; this method can be used to attach oligonucleotides to nanoparticles). The alkanethiol method can also be used to attach oligonucleotides to other metal, semiconductor and magnetic colloids and to the other nanoparticles listed above. Other functional groups for attaching oligonucleotides to solid surfaces include phosphorothioate groups (see, _e.g._, U.S. Patent No. 5,472,881 for the binding of oligonucleotide-phosphorothioates to gold surfaces), substituted alkylsiloxanes (see, _e.g._ Burwell, _Chemical Technology_, 4, 370-377 (1974) and Matteucci and Caruthers, _J. Am. Chem. Soc._, 103, 3185-3191 (1981) for binding of oligonucleotides to silica and glass surfaces, and Grabar et al., _Anal. Chem._, 67, 735-743 for binding of aminoalkylsiloxanes and for similar binding of mercaptoaklylsiloxanes). Oligonucleotides terminated with a 5' thionucleoside or a 3' thionucleoside may also

22

be used for attaching oligonucleotides to solid surfaces.
Gold nanoparticles may be attached to oligonucleotides
using biotin-labeled oligonucleotides and streptavidin-
gold conjugate colloids; the biotin-streptavidin

5   interaction attaches the colloids to the oligonucleotide.
Shaiu et al., *Nuc. Acids Res.*, **21**, 99 (1993). The
following references describe other methods which may be
employed to attached oligonucleotides to nanoparticles:
Nuzzo et al., *J. Am. Chem. Soc.*, **109**, 2358 (1987)

10   (disulfides on gold); Allara and Nuzzo, *Langmuir*, **1**, 45
(1985) (carboxylic acids on aluminum); Allara and
Tompkins, *J. Colloid Interface Sci.*, **49**, 410-421 (1974)
(carboxylic acids on copper); Iler, The Chemistry Of
Silica, Chapter 6, (Wiley 1979) (carboxylic acids on

15   silica); Timmons and Zisman, *J. Phys. Chem.*, **69**, 984-990
(1965) (carboxylic acids on platinum); Soriaga and
Hubbard, *J. Am. Chem. Soc.*, **104**, 3937 (1982) (aromatic
ring compounds on platinum); Hubbard, *Acc. Chem. Res.*,
13, 177 (1980) (sulfolanes, sulfoxides and other

20   functionalized solvents on platinum); Hickman et al., *J.
Am. Chem. Soc.*, **111**, 7271 (1989) (isonitriles on
platinum); Maoz and Sagiv, *Langmuir*, **3**, 1045 (1987)
(silanes on silica); Maoz and Sagiv, *Langmuir*, **3**, 1034
(1987) (silanes on silica); Wasserman et al., *Langmuir*,

25   **5**, 1074 (1989) (silanes on silica); Eltekova and Eltekov,
Langmuir, **3**, 951 (1987) (aromatic carboxylic acids,
aldehydes, alcohols and methoxy groups on titanium
dioxide and silica); Lec et al., *J. Phys. Chem.*, **92**, 2597
(1988) (rigid phosphates on metals).

30   Each nanoparticle will have a plurality of
oligonucleotides attached to it. As a result, each
nanoparticle-oligonucleotide conjugate can bind to a
plurality of oligonucleotides or nucleic acids having the
complementary sequence.

23

Oligonucleotides of defined sequences are used for a variety of purposes in the practice of the invention. Methods of making oligonucleotides of a predetermined sequence are well-known. See, *e.g.*, Sambrook *et al.*,

5   *Molecular Cloning: A Laboratory Manual* (2nd ed. 1989) and F. Eckstein (ed.) *Oligonucleotides and Analogues*, 1st Ed. (Oxford University Press, New York, 1991). Solid-phase synthesis methods are preferred for both oligoribonucleotides and oligodeoxyribonucleotides (the

10   well-known methods of synthesizing DNA are also useful for synthesizing RNA). Oligoribonucleotides and oligodeoxyribonucleotides can also be prepared enzymatically.

The invention provides methods of detecting nucleic

15   acids. Any type of nucleic acid may be detected, and the methods may be used, *e.g.*, for the diagnosis of disease and in sequencing of nucleic acids. Examples of nucleic acids that can be detected by the methods of the invention include genes (*e.g.*, a gene associated with a

20   particular disease), viral RNA and DNA, bacterial DNA, fungal DNA, cDNA, mRNA, RNA and DNA fragments, oligonucleotides, synthetic oligonucleotides, modified oligonucleotides, single-stranded and double-stranded nucleic acids, natural and synthetic nucleic acids, etc.

25   Thus, examples of the uses of the methods of detecting nucleic acids include: the diagnosis and/or monitoring of viral diseases (*e.g.*, human immunodeficiency virus, hepatitis viruses, herpes viruses, cytomegalovirus, and Epstein-Barr virus), bacterial diseases (*e.g.*,

30   tuberculosis, Lyme disease, *H. pylori*, *Escherichia coli* infections, *Legionella* infections, *Mycoplasma* infections, *Salmonella* infections), sexually transmitted diseases (*e.g.*, gonorrhoea), inherited disorders (*e.g.*, cystic fibrosis, Duchene muscular dystrophy, phenylketonuria,

sickle cell anemia), and cancers (e.g., genes associated with the development of cancer); in forensics; in DNA sequencing; for paternity testing; for cell line authentication; for monitoring gene therapy; and for many
5   other purposes.

The methods of detecting nucleic acids based on observing a color change with the naked eye are cheap, fast, simple, robust (the reagents are stable), do not require specialized or expensive equipment, and do not
10   require any instrumentation. This makes them particularly suitable for use in, e.g., research and analytical laboratories in DNA sequencing, in the field to detect the presence of specific pathogens, in the doctor's office for quick identification of an infection
15   to assist in prescribing a drug for treatment, and in homes and health centers for inexpensive first-line screening.

The nucleic acid to be detected may be isolated by known methods, or may be detected directly in cells,
20   tissue samples, biological fluids (e.g., saliva, urine, blood, serum), solutions containing PCR components, solutions containing large excesses of oligonucleotides or high molecular weight DNA, and other samples, as also known in the art. See, e.g., Sambrook et al., Molecular
25   Cloning: A Laboratory Manual (2nd ed. 1989) and B.D. Hames and S.J. Higgins, Eds., Gene Probes 1 (IRL Press, New York, 1995). Methods of preparing nucleic acids for detection with hybridizing probes are well known in the art. See, e.g., Sambrook et al., Molecular Cloning: A
30   Laboratory Manual (2nd ed. 1989) and B.D. Hames and S.J. Higgins, Eds., Gene Probes 1 (IRL Press, New York, 1995).

If a nucleic acid is present in small amounts, it may be amplifed by methods known in the art. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual

(2nd ed. 1989) and B.D. Hames and S.J. Higgins, Eds., *Gene Probes 1* (IRL Press, New York, 1995). Preferred is polymerase chain reaction (PCR) amplification.

One method according to the invention for detecting
5 nucleic acid comprises contacting a nucleic acid with one or more types of nanoparticles having oligonucleotides attached thereto. The nucleic acid to be detected has at least two portions. The lengths of these portions and the distance(s), if any, between them are chosen so that
10 when the oligonucleotides on the nanoparticles hybridize to the nucleic acid, a detectable change occurs. These lengths and distances can be determined empirically and will depend on the type of particle used and its size and the type of electrolyte which will be present in
15 solutions used in the assay (certain electrolytes affect the conformation of nucleic acids).

Also, when a nucleic acid is to be detected in the presence of other nucleic acids, the portions of the nucleic acid to which the oligonucleotides on the
20 nanoparticles are to bind must be chosen so that they contain sufficient unique sequence so that detection of the nucleic acid will be specific. Guidelines for doing so are well known in the art.

Although nucleic acids may contain repeating
25 sequences close enough to each other so that only one type of oligonucleotide-nanoparticle conjugate need be used, this will be a rare occurrence. In general, the chosen portions of the nucleic acid will have different sequences and will be contacted with nanoparticles
30 carrying two or more different oligonucleotides, preferably attached to different nanoparticles. An example of a system for the detection of nucleic acid is illustrated in Figure 2. As can be seen, a first oligonucleotide attached to a first nanoparticle has a
35 sequence complementary to a first portion of the target

26

sequence in the single-stranded DNA. A second
oligonucleotide attached to a second nanoparticle has a
sequence complementary to a second portion of the target
sequence in the DNA. Additional portions of the DNA

5   could be targeted with corresponding nanoparticles. See
Figure 17. Targeting several portions of a nucleic acid
increases the magnitude of the detectable change.

The contacting of the nanoparticle-oligonucleotide
conjugates with the nucleic acid takes place under

10 conditions effective for hybridization of the
oligonucleotides on the nanoparticles with the target
sequence(s) of the nucleic acid. These hybridization
conditions are well known in the art and can readily be
optimized for the particular system employed. See, *e.g.*,

15 Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*
(2nd ed. 1989). Preferably stringent hybridization
conditions are employed.

Faster hybridization can be obtained by freezing and
thawing a solution containing the nucleic acid to be

20 detected and the nanoparticle-oligonucleotide conjugates.
The solution may be frozen in any convenient manner, such
as placing it in a dry ice-alcohol bath for a sufficient
time for the solution to freeze (generally about 1 minute
for 100 µL of solution). The solution must be thawed at

25 a temperature below the thermal denaturation temperature,
which can conveniently be room temperature for most
combinations of nanoparticle-oligonucleotide conjugates
and nucleic acids. The hybridization is complete, and
the detectable change may be observed, after thawing the

30 solution.

The rate of hybridization can also be increased by
warming the solution containing the nucleic acid to be
detected and the nanoparticle-oligonucleotide conjugates
to a temperature below the dissociation temperature (Tm)

35 for the complex formed between the oligonucleotides on

the nanoparticles and the target nucleic acid. Alternatively, rapid hybridization can be achieved by heating above the dissociation temperature (Tm) and allowing the solution to cool.

5   The rate of hybridization can also be increased by increasing the salt concentration (e.g., from 0.1 M to 0.3 M NaCl).

        The detectable change that occurs upon hybridization of the oligonucleotides on the nanoparticles to the
10   nucleic acid may be a color change, the formation of aggregates of the nanoparticles, or the precipitation of the aggregated nanoparticles. The color changes can be observed with the naked eye or spectroscopically. The formation of aggregates of the nanoparticles can be
15   observed by electron microscopy or by nephelometry. The precipitation of the aggregated nanoparticles can be observed with the naked eye or microscopically. Preferred are changes observable with the naked eye. Particularly preferred is a color change observable with
20   the naked eye.

        The observation of a color change with the naked eye can be made more readily against a background of a contrasting color. For instance, when gold nanoparticles are used, the observation of a color change is
25   facilitated by spotting a sample of the hybridization solution on a solid white surface (such as silica or alumina TLC plates, filter paper, cellulose nitrate membranes, and nylon membranes, preferably a C-18 silica TLC plate) and allowing the spot to dry. Initially, the
30   spot retains the color of the hybridization solution (which ranges from pink/red, in the absence of hybridization, to purplish-red/purple, if there has been hybridization). On drying at room temperature or 80°C (temperature is not critical), a blue spot develops if
35   the nanoparticle-oligonucleotide conjugates had been

linked by hybridization with the target nucleic acid
prior to spotting. In the absence of hybridization
(e.g., because no target nucleic acid is present), the
spot is pink. The blue and the pink spots are stable and
5   do not change on subsequent cooling or heating or over
time. They provide a convenient permanent record of the
test. No other steps (such as a separation of hybridized
and unhybridized nanoparticle-oligonucleotide conjugates)
are necessary to observe the color change.
10   An alternate method for easily visualizing the assay
results would be to spot a sample of nanoparticle probes
hybridized to a target nucleic acid on a glass fiber
filter (e.g., Borosilicate Microfiber Filter, 0.7 micron
pore size, grade FG75, for use with gold nanoparticles 13
15   nm in size), while drawing the liquid through the filter.
Subsequent rinsing with water washes the excess, non-
hybridized probes through the filter, leaving behind an
observable spot comprising the aggregates generated by
hybridization of the nanoparticle probes with the target
20   nucleic acid (retained because these aggregates are
larger than the pores of the filter). This technique may
provide for greater sensitivity, since an excess of
nanoparticle probes can be used. Unfortunately, the
nanoparticle probes stick to all other solid surfaces
25   that have been tried (silica slides, reverse-phase
plates, and nylon, nitrocellulose, cellulose and other
membranes), making glass fiber filters the only solid
surface known to date which can be be employed in such a
washing format.
30   An important aspect of the detection system
illustrated in Figure 2 is that obtaining a detectable
change depends on cooperative hybridization of two
different oligonucleotides to a given target sequence in
the nucleic acid. Mismatches in either of the two
35   oligonucleotides will destabilize the interparticle

connection. It is well known that a mismatch in base
pairing has a much greater destabilizing effect on the
binding of a short oligonucleotide probe than on the
binding of a long oligonucleotide probe. The advantage
5   of the system illustrated in Figure 2 is that it utilizes
the base discrimination associated with a long target
sequence and probe (eighteen base-pairs in the example
illustrated in Figure 2), yet has the sensitivity
characteristic of a short oligonucleotide probe (nine
10 base-pairs in the example illustrated in Figure 2).

The target sequence of the nucleic acid may be
contiguous, as in Figure 2, or the two portions of the
target sequence may be separated by a third portion which
is not complementary to the oligonucleotides on the
15 nanoparticles, as illustrated in Figure 3. In the latter
case, one has the option of using a filler
oligonucleotide which is free in solution and which has a
sequence complementary to that of this third portion (see
Figure 3). When the filler oligonucleotide hybridizes
20 with the third portion of the nucleic acid, a double-
stranded segment is created, thereby altering the average
distance between the nanoparticles and, consequently, the
color. The system illustrated in Figure 3 may increase
the sensitivity of the detection method.

25   Some embodiments of the method of detecting nucleic
acid utilize a substrate. By employing a substrate, the
detectable change (the signal) can be amplified and the
sensitivity of the assay increased.

Any substrate can be used which allows observation
30 of the detectable change. Suitable substrates include
transparent solid surfaces (e.g., glass, quartz, plastics
and other polymers), opaque solid surface (e.g., white
solid surfaces, such as TLC silica plates, filter paper,
glass fiber filters, cellulose nitrate membranes, nylon
35 membranes), and conducting solid surfaces (e.g., indium-

tin-oxide (ITO)). The substrate can be any shape or
thickness, but generally will be flat and thin.
Preferred are transparent substrates such as glass (e.g.,
glass slides) or plastics (e.g., wells of microtiter
5   plates).

In one embodiment, oligonucleotides are attached to
the substrate. The oligonucleotides can be attached to
the substrates as described in, e.g., Chrisey et al.,
Nucleic Acids Res., **24**, 3031-3039 (1996); Chrisey et al.,
10   *Nucleic Acids Res.*, **24**, 3040-3047 (1996); Mucic et al.,
Chem. Commun., **555** (1996); Zimmermann and Cox, *Nucleic
Acids Res.*, **22**, 492 (1994); Bottomley et al., *J. Vac.
Sci. Technol. A*, **10**, 591 (1992); and Hegner et al., *FEBS
Lett.*, **336**, 452 (1993).

15   The oligonucleotides attached to the substrate have
a sequence complementary to a first portion of the
sequence of the nucleic acid to be detected. The nucleic
acid is contacted with the substrate under conditions
effective to allow hybridization of the oligonucleotides
20   on the substrate with the nucleic acid. In this manner
the nucleic acid becomes bound to the substrate. Any
unbound nucleic acid is preferably washed from the
substrate before adding nanoparticle-oligonucleotide
conjugates.

25   Next, the nucleic acid bound to the substrate is
contacted with a first type of nanoparticles having
oligonucleotides attached thereto. The oligonucleotides
have a sequence complementary to a second portion of the
sequence of the nucleic acid, and the contacting takes
30   place under conditions effective to allow hybridization
of the oligonucleotides on the nanoparticles with the
nucleic acid. In this manner the first type of
nanoparticles become bound to the substrate. After the
nanoparticle-oligonucleotide conjugates are bound to the

substrate, the substrate is washed to remove any unbound nanoparticle-oligonucleotide conjugates and nucleic acid.

The oligonucleotides on the first type of nanoparticles may all have the same sequence or may have

5   different sequences that hybridize with different portions of the nucleic acid to be detected. When oligonucleotides having different sequences are used, each nanoparticle may have all of the different oligonucleotides attached to it or, preferably, the

10   different oligonucleotides are attached to different nanoparticles. Figure 17 illustrates the use of nanoparticle-oligonucleotide conjugates designed to hybridize to multiple portions of a nucleic acid.

Finally, the first type of nanoparticle-
15   oligonucleotide conjugates bound to the substrate is contacted with a second type of nanoparticles having oligonucleotides attached thereto. These oligonucleotides have a sequence complementary to at least a portion of the sequence(s) of the

20   oligonucleotides attached to the first type of nanoparticles, and the contacting takes place under conditions effective to allow hybridization of the oligonucleotides on the first type of nanoparticles with those on the second type of nanoparticles. After the

25   nanoparticles are bound, the substrate is preferably washed to remove any unbound nanoparticle-oligonucleotide conjugates.

The combination of hybridizations produces a detectable change. The detectable changes are the same

30   as those described above, except that the multiple hybridizations result in an amplification of the detectable change. In particular, since each of the first type of nanoparticles has multiple oligonucleotides (having the same or different sequences) attached to it,

35   each of the first type of nanoparticle-oligonucleotide

conjugates can hybridize to a plurality of the second
type of nanoparticle-oligonucleotide conjugates. Also,
the first type of nanoparticle-oligonucleotide conjugates
may be hybridized to more than one portion of the nucleic

5   acid to be detected. The amplification provided by the
multiple hybridizations may make the change detectable
for the first time or may increase the magnitude of the
detectable change. This amplification increases the
sensitivity of the assay, allowing for detection of small

10 amounts of nucleic acid.

If desired, additional layers of nanoparticles can
be built up by successive additions of the first and
second types of nanoparticle-oligonucleotide conjugates.
In this way, the number of nanoparticles immobilized per

15 molecule of target nucleic acid can be further increased
with a corresponding increase in intensity of the signal.

Also, instead of using first and second types of
nanoparticle-oligonucleotide conjugates designed to
hybridize to each other directly, nanoparticles bearing

20 oligonucleotides that would serve to bind the
nanoparticles together as a consequence of hybridization
with binding oligoncleotides could be used.

Methods of making the nanoparticles and the
oligonucleotides and of attaching the oligonucleotides to

25 the nanoparticles are described above. The hybridization
conditions are well known in the art and can be readily
optimized for the particular system employed (see above).

An example of this method of detecting nucleic acid
(analyte DNA) is illustrated in Figure 13A. The

30 combination of hybridizations produces dark areas where
nanoparticle aggregates are linked to the substrate by
analyte DNA. These dark areas may be readily observed
with the naked eye using ambient light, preferably
viewing the substrate against a white background. As can

be readily seen from Figure 13A, this method provides a means of amplifying a detectable change.

In another embodiment, nanoparticles are attached to the substrate. Nanoparticles can be attached to

5   substrates as described in, e.g., Grabar et al., *Analyt.
Chem., **67**, 73-743 (1995); Bethell et al., *J. Electroanal. Chem.*, **409**, 137 (1996); Bar et al., *Langmuir*, **12**, 1172 (1996); Colvin et al., *J. Am. Chem. Soc.*, **114**, 5221 (1992).

10   After the nanoparticles are attached to the substrate, oligonucleotides are attached to the nanoparticles. This may be accomplished in the same manner described above for the attachment of oligonucleotides to nanoparticles in solution. The

15   oligonucleotides attached to the nanoparticles have a sequence complementary to a first portion of the sequence of a nucleic acid.

The substrate is contacted with the nucleic acid under conditions effective to allow hybridization of the

20   oligonucleotides on the nanoparticles with the nucleic acid. In this manner the nucleic acid becomes bound to the substrate. Unbound nucleic acid is preferably washed from the substrate prior to adding further nanoparticle-oligonucleotide conjugates.

25   Then, a second type of nanoparticles having oligonucleotides attached thereto is provided. These oligonucleotides have a sequence complementary to a second portion of the sequence of the nucleic acid, and the nucleic acid bound to the substrate is contacted with

30   the second type of nanoparticle-oligonucleotide conjugates under conditions effective to allow hybridization of the oligonucleotides on the second type of nanoparticle-oligonucleotide conjugates with the nucleic acid. In this manner, the second type of

35   nanoparticle-oligonucleotide conjugates becomes bound to

the substrate. After the nanoparticles are bound, any unbound nanoparticle-oligonucleotide conjugates and nucleic acid are washed from the substrate. A change (e.g., color change) may be detectable at this point.

5   The oligonucleotides on the second type of nanoparticles may all have the same sequence or may have different sequences that hybridize with different portions of the nucleic acid to be detected. When oligonucleotides having different sequences are used,

10   each nanoparticle may have all of the different oligonucleotides attached to it or, preferably, the different oligonucleotides may be attached to different nanoparticles. See Figure 17.

       Next, a binding oligonucleotide having a selected

15   sequence having at least two portions, the first portion being complementary to at least a portion of the sequence of the oligonucleotides on the second type of nanoparticles, is contacted with the second type of nanoparticle-oligonucleotide conjugates bound to the

20   substrate under conditions effective to allow hybridization of the binding oligonucleotide to the oligonucleotides on the nanoparticles. In this manner, the binding oligonucleotide becomes bound to the substrate. After the binding oligonucleotides are bound,

25   unbound binding oligonucleotides are washed from the substrate.

       Finally, a third type of nanoparticles having oligonucleotides attached thereto is provided. The oligonucleotides have a sequence complementary to the

30   sequence of a second portion of the binding oligonucleotide. The nanoparticle-oligonucleotide conjugates are contacted with the binding oligonucleotide bound to the substrate under conditions effective to allow hybridization of the binding oligonucleotide to the

35   oligonucleotides on the nanoparticles. After the

nanoparticles are bound, unbound nanoparticle-
oligonucleotide conjugates are washed from the substrate.

The combination of hybridizations produces a
detectable change. The detectable changes are the same

5   as those described above, except that the multiple
hybridizations result in an amplification of the
detectable change. In particular, since each of the
second type of nanoparticles has multiple
oligonucleotides (having the same or different sequences)

10 attached to it, each of the second type of nanoparticle-
oligonucleotide conjugates can hybridize to a plurality
of the third type of nanoparticle-oligonucleotide
conjugates (through the binding oligonucleotide). Also,
the second type of nanoparticle-oligonucleotide

15 conjugates may be hybridized to more than one portion of
the nucleic acid to be detected. The amplification
provided by the multiple hybridizations may make the
change detectable for the first time or may increase the
magnitude of the detectable change. The amplification

20 increases the sensitivity of the assay, allowing for
detection of small amounts of nucleic acid.

If desired, additional layers of nanoparticles can
be built up by successive additions of the binding
oligonucleotides and second and third types of

25 nanoparticle-oligonucleotide conjugates. In this way,
the nanoparticles immobilized per molecule of target
nucleic acid can be further increased with a
corresponding increase in intensity of the signal.

Also, the use of the binding oligonucleotide can be

30 eliminated, and the second and third types of
nanoparticle-oligonucleotide conjugates can be designed
so that they hybridize directly to each other.

Methods of making the nanoparticles and the
oligonucleotides and of attaching the oligonucleotides to

35 the nanoparticles are described above. The hybridization

conditions are well known in the art and can be readily optimized for the particular system employed (see above).

An example of this method of detecting nucleic acid (analyte DNA) is illustrated in Figure 13B. The

5 combination of hybridizations produces dark areas where nanoparticle aggregates are linked to the substrate by analyte DNA. These dark areas may be readily observed with the naked eye as described above. As can be seen from Figure 13B, this embodiment of the method of the

10 invention provides another means of amplifying the detectable change.

Another amplification scheme employs liposomes. In this scheme, oligonucleotides are attached to a substrate. Suitable substrates are those described

15 above, and the oligonucleotides can be attached to the substrates as described above. For instance, where the substrate is glass, this can be accomplished by condensing the oligonucleotides through phosphoryl or carboxylic acid groups to aminoalkyl groups on the

20 substrate surface (for related chemistry see Grabar et al., *Anal. Chem.*, **67**, 735-743 (1995)).

The oligonucleotides attached to the substrate have a sequence complementary to a first portion of the sequence of the nucleic acid to be detected. The nucleic

25 acid is contacted with the substrate under conditions effective to allow hybridization of the oligonucleotides on the substrate with the nucleic acid. In this manner the nucleic acid becomes bound to the substrate. Any unbound nucleic acid is preferably washed from the

30 substrate before adding additional components of the system.

Next, the nucleic acid bound to the substrate is contacted with liposomes having oligonucleotides attached thereto. The oligonucleotides have a sequence

35 complementary to a second portion of the sequence of the

37

nucleic acid, and the contacting takes place under
conditions effective to allow hybridization of the
oligonucleotides on the liposomes with the nucleic acid.
In this manner the liposomes become bound to the
5   substrate. After the liposomes are bound to the
substrate, the substrate is washed to remove any unbound
liposomes and nucleic acid.

     The oligonucleotides on the liposomes may all have
the same sequence or may have different sequences that
10   hybridize with different portions of the nucleic acid to
be detected. When oligonucleotides having different
sequences are used, each liposome may have all of the
different oligonucleotides attached to it or the
different oligonucleotides may be attached to different
15   liposomes.

     To prepare oligonucleotide-liposome conjugates, the
oligonucleotides are linked to a hydrophobic group, such
as cholesteryl (see Letsinger et al., *J. Am. Chem. Soc.*,
115, 7535-7536 (1993)), and the hydrophobic-
20   oligonucleotide conjugates are mixed with a solution of
liposomes to form liposomes with hydrophobic-
oligonucleotide conjugates anchored in the membrane (see
Zhang et al., *Tetrahedron Lett.*, **37**, 6243-6246 (1996)).
The loading of hydrophobic-oligonucleotide conjugates on
25   the surface of the liposomes can be controlled by
controlling the ratio of hydrophobic-oligonucleotide
conjugates to liposomes in the mixture. It has been
observed that liposomes bearing oligonucleotides attached
by hydrophobic interaction of pendent cholesteryl groups
30   are effective in targeting polynucleotides immobilized on
a nitrocellulose membrane (*Id.*). Fluorescein groups
anchored in the membrane of the liposome were used as the
reporter group. They served effectively, but sensitivity
was limited by the fact that the signal from fluorescein

in regions of high local concentration (e.g., on the liposome surface) is weakened by self quenching.

The liposomes are made by methods well known in the art. See Zhang et al., *Tetrahedron Lett.*, **37**, 6243

5   (1996). The liposomes will generally be about 5-50 times the size (diameter) of the nanoparticles used in subsequent steps. For instance, for nanoparticles about 13 nm in diameter, liposomes about 100 nm in diameter are preferably used.

10   The liposomes bound to the substrate are contacted with a first type of nanoparticles having at least a first type of oligonucleotides attached thereto. The first type of oligonucleotides have a hydrophobic group attached to the end not attached to the nanoparticles,

15   and the contacting takes place under conditions effective to allow attachment of the oligonucleotides on the nanoparticles to the liposomes as a result of hydrophobic interactions. A detectable change may be observable at this point.

20   The method may further comprise contacting the first type of nanoparticle-oligonucleotide conjugates bound to the liposomes with a second type of nanoparticles having oligonucleotides attached thereto. The first type of nanoparticles have a second type of oligonucleotides

25   attached thereto which have a sequence complementary to at least a portion of the sequence of the oligonucleotides on the second type of nanoparticles, and the oligonucleotides on the second type of nanoparticles have a sequence complementary to at least a portion of

30   the sequence of the second type of oligonucleotides on the first type of nanoparticles. The contacting takes place under conditions effective to allow hybridization of the oligonucleotides on the first and second types of nanoparticles. This hybridization will generally be

35   performed at mild temperatures (e.g., 5°C to 60°C), so

conditions (*e.g.*, 0.3-1.0 M NaCl) conducive to hybridization at room temperature are employed. Following hybridization, unbound nanoparticle-oligonucleotide conjugates are washed from the substrate.

5   The combination of hybridizations produces a detectable change. The detectable changes are the same as those described above, except that the multiple hybridizations result in an amplification of the detectable change. In particular, since each of the liposomes has multiple oligonucleotides (having the same or different sequences) attached to it, each of the liposomes can hybridize to a plurality of the first type of nanoparticle-oligonucleotide conjugates. Similarly, since each of the first type of nanoparticles has multiple oligonucleotides attached to it, each of the first type of nanoparticle-oligonucleotide conjugates can hybridize to a plurality of the second type of nanoparticle-oligonucleotide conjugates. Also, the liposomes may be hybridized to more than one portion of the nucleic acid to be detected. The amplification provided by the multiple hybridizations may make the change detectable for the first time or may increase the magnitude of the detectable change. This amplification increases the sensitivity of the assay, allowing for detection of small amounts of nucleic acid.

If desired, additional layers of nanoparticles can be built up by successive additions of the first and second types of nanoparticle-oligonucleotide conjugates. In this way, the number of nanoparticles immobilized per molecule of target nucleic acid can be further increased with a corresponding increase in the intensity of the signal. Further enhancement can also be obtained by employing silver staining of gold nanoparticles (Bassell, et al., *J. Cell Biol.*, **126**, 863-876 (1994); Braun-Howland et al., *Biotechniques*, **13**, 928-931 (1992)).

40

Also, instead of using second and third types of
nanoparticle-oligonucleotide conjugates designed to
hybridize to each other directly, nanoparticles bearing
oligonucleotides that would serve to bring the
5   nanoparticles together as a consequence of hybridization
with binding oligonucleotides could be used.

Methods of making the nanoparticles and the
oligonucleotides and of attaching the oligonucleotides to
the nanoparticles are described above. A mixture of
10 oligonucleotides functionalized at one end for binding to
the nanoparticles and with or without a hydrophobic group
at the other end can be used on the first type of
nanoparticles. The relative ratio of these
oligonucleotides bound to the average nanoparticle will
15 be controlled by the ratio of the concentrations of the
two oligonucleotides in the mixture. The hybridization
conditions are well known in the art and can be readily
optimized for the particular system employed (see above).

An example of this method of detecting nucleic acid
20 is illustrated in Figure 18. The hybridization of the
first type of nanoparticle-oligonucleotide conjugates to
the liposomes may produce a detectable change. In the
case of gold nanoparticles, a pink/red color may be
observed or a purple/blue color may be observed if the
25 nanoparticles are close enough together. The
hybridization of the second type of nanoparticle-
oligonucleotide conjugates to the first type of
nanoparticle-oligonucleotide conjugates will produce a
detectable change. In the case of gold nanoparticles, a
30 purple/blue color will be observed. All of these color
changes may be observed with the naked eye.

When a substrate is employed, a plurality of the
initial types of nanoparticle-oligonucleotide conjugates
or oligonucleotides can be attached to the substrate in
35 an array for detecting multiple portions of a target

nucleic acid, for detecting multiple different nucleic
acids, or both. For instance, a substrate may be
provided with rows of spots, each spot containing a
different type of oligonucleotide or oligonucleotide-
5   nanoparticle conjugate designed to bind to a portion of a
target nucleic acid. A sample containing one or more
nucleic acids is applied to each spot, and the rest of
the assay is performed in one of the ways described above
using appropriate oligonucleotide-nanoparticle
10   conjugates, oligonucleotide-liposome conjugates and
binding oligonucleotides.

A nanoparticle-oligonucleotide conjugate which may
be used in an assay for any nucleic acid is illustrated
in Figure 17, parts IV and V. This "universal probe" has
15   oligonucleotides of a single sequence attached to it.
These oligonucleotides can hybridize with a binding
oligonucleotide which has a sequence comprising at least
two portions. The first portion is complementary to at
least a portion of the sequence of the oligonucleotides
20   on the nanoparticles. The second portion is
complementary to a portion of the sequence of the nucleic
acid to be detected. A plurality of binding
oligonucleotides having the same first portion and
different second portions can be used, in which case the
25   "universal probe", after hybridization to the binding
oligonucleotides, can bind to multiple portions of the
nucleic acid to be detected or to different nucleic acid
targets.

In yet another embodiment, oligonucleotides attached
30   to metal and semiconductor nanoparticles can have a
fluorescent molecule attached to the end not attached to
the nanoparticles. Metal and semiconductor nanoparticles
are known fluorescence quenchers, with the magnitude of
the quenching effect depending on the distance between
35   the nanoparticles and the fluorescent molecule. In the

unhybridized state, the oligonucleotides attached to the nanoparticles interact with the nanoparticles, so that significant quenching will be observed. See Figure 20A. Upon hybridization to a target nucleic acid, the

5   fluorescent molecule will become spaced away from the nanoparticles, diminishing quenching of the fluorescence. See Figure 20A. Longer oligonucleotides should give rise to larger changes in fluorescence, at least until the fluorescent groups are moved far enough away from the

10   nanoparticle surfaces so that an increase in the change is no longer observed. Useful lengths of the oligonucleotides can be determined empirically. Metallic and semiconductor nanoparticles having fluorescent-labeled oligonucleotides attached thereto can be used in

15   any of the assay formats described above, including those performed in solution or on substrates.

Methods of labeling oligonucleotides with fluorescent molecules and measuring fluorescence are well known in the art. Suitable fluorescent molecules are

20   also well known in the art and include the fluoresceins, rhodamines and Texas Red. The oligonucleotides will be attached to the nanoparticles as described above.

In yet another embodiment, two types of fluorescent-labeled oligonucleotides attached to two

25   different particles can be used. Suitable particles include polymeric particles, such as polystyrene particles, polyvinyl particles, acrylate and methacrylate particles, glass particles, latex particles, Sepharose beads and others like particles well known in the art.

30   Methods of attaching oligonucleotides to such particles are well known in the art. In particular, a wide variety of functional groups are availble on the particles or can be incorporated into such particles. Functional groups include carboxylic acids, aldehydes, amino groups, cyano

35   groups, ethylene groups, hydroxyl groups, mercapto

43

groups, and the like. Nanoparticles, including metallic and semiconductor nanoparticles can also be used.

The two fluorophores are designated **d** and **a** for donor and acceptor. A variety of fluorescent molecules
5   useful in such combinations are well known in the art and are available from, *e.g.*, Molecular Probes. An attractive combination is fluorescein as the donor and Texas Red as acceptor. The two types of nanoparticle-oligonucleotide conjugates with **d** and **a** attached are
10   mixed with the target nucleic acid, and fluorescence measured in a fluorimeter. The mixture will be excited with light of the wavelength that excites **d**, and the mixture will be monitored for fluorescence from **a**. Upon hybridization, **d** and **a** will be brought in proximity (see
15   Figure 20B).

In the case of non-metallic, non-semiconductor particles, hybridization will be shown by a shift in fluorescence from that for **d** to that for **a** or by the appearance of fluorescence for **a** in addition to
20   that for **d**. In the absence of hybridization, the flurophores will be too far apart for energy transfer to be significant, and only the fluorescence of **d** will be observed.

In the case of metallic and semiconductor
25   nanoparticles, lack of hybridization will be shown by a lack of fluorescence due to **d** or **a** because of quenching (see above). Hybridization will be shown by an increase in fluorescence due to **a**.

As will be appreciated, the above described
30   particles and nanoparticles having oligonucleotides labeled with acceptor and donor fluorescent molecules attached can be be used in the assay formats described above, including those performed in solution and on substrates. For solution formats, the oligonucleotide

sequences are preferably chosen so that they bind to the
target nucleic acid as illustrated in Figure 15. In the
formats shown in Figure 13A-B and 18, the binding
oligonucleotides may be used to bring the acceptor and

5   donor fluorescent molecules on the two nanoparticles in
proximity. Also, in the format illustrated in Figure
13A, the oligonucleotides attached the substrate may be
labeled with **d**. Further, in principle, other labels
besides fluorescent molecules can be used, such as

10   chemiluminescent molecules, which will give a detectable
signal or a change in detectable signal upon
hybridization.

        . The invention also provides kits for detecting
nucleic acids. In one embodiment, the kit comprises at

15   least one container, the container holding at least two
types of nanoparticles having oligonucleotides attached
thereto. The oligonucleotides on the first type of
nanoparticles have a sequence complementary to the
sequence of a first portion of a nucleic acid. The

20   oligonucleotides on the second type of nanoparticles have
a sequence complementary to the sequence of a second
portion of the nucleic acid. The container may further
comprise filler oligonucleotides having a sequence
complementary to a third portion of the nucleic acid, the

25   third portion being located between the first and second
portions. The filler oligonucleotide may also be
provided in a separate container.

        In a second embodiment, the kit comprises at least
two containers. The first container holds nanoparticles

30   having oligonucleotides attached thereto which have a
sequence complementary to the sequence of a first portion
of a nucleic acid. The second container holds
nanoparticles having oligonucleotides attached thereto
which have a sequence complementary to the sequence of a

35   second portion of the nucleic acid. The kit may further

comprise a third container holding a filler oligonucleotide having a sequence complementary to a third portion of the nucleic acid, the third portion being located between the first and second portions.

5   In another alternative embodiment, the kits can have the oligonucleotides and nanoparticles in separate containers, and the oligonucleotides would have to be attached to the nanoparticles prior to performing an assay to detect a nucleic acid. The oligonucleotides

10   and/or the nanoparticles may be functionalized so that the oligonucleotides can be attached to the nanoparticles. Alternatively, the oligonucleotides and/or nanoparticles may be provided in the kit without functional groups, in which case they must be

15   functionalized prior to performing the assay.

        In another embodiment, the kit comprises at least one container. The container holds metallic or semiconductor nanoparticles having oligonucleotides attached thereto. The oligonucleotides have a sequence

20   complementary to a portion of a nucleic acid and have fluorescent molecules attached to the ends of the oligonucleotides not attached to the nanoparticles.

        In yet another embodiment, the kit comprises a substrate, the substrate having attached thereto

25   nanoparticles. The nanoparticles have oligonucleotides attached thereto which have a sequence complementary to the sequence of a first portion of a nucleic acid. The kit also includes a first container holding nanoparticles having oligonucleotides attached thereto which have a

30   sequence complementary to the sequence of a second portion of the nucleic acid. The oligonucleotides may have the same or different sequences, but each of the oligonucleotides has a sequence complementary to a portion of the nucleic acid. The kit further includes a

35   second container holding a binding oligonucleotide having

a selected sequence having at least two portions, the first portion being complementary to at least a portion of the sequence of the oligonucleotides on the nanoparticles in the first container. The kit also

5 includes a third container holding nanoparticles having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to the sequence of a second portion of the binding oligonucleotide.

In another embodiment, the kit comprises a substrate

10 having oligonucleotides attached thereto which have a sequence complementary to the sequence of a first portion of a nucleic acid. The kit also includes a first container holding nanoparticles having oligonucleotides attached thereto which have a sequence complementary to

15 the sequence of a second portion of the nucleic acid. The oligonucleotides may have the same or different sequences, but each of the oligonucleotides has a sequence complementary to a portion of the nucleic acid. The kit further includes a second container holding

20 nanoparticles having oligonucleotides attached thereto which have a sequence complementary to at least a portion of the oligonucleotides attached to the nanoparticles in the first container.

In yet another embodiment, the kits can have the

25 substrate, oligonucleotides and nanoparticles in separate containers. The substrate, oligonucleotides, and nanoparticles would have to be appropriately attached to each other prior to performing an assay to detect a nucleic acid. The substrate, oligonucleotides and/or the

30 nanoparticles may be functionalized to expedite this attachment. Alternatively, the substrate, oligonucleotides and/or nanoparticles may be provided in the kit without functional groups, in which case they must be functionalized prior to performing the assay.

In a further embodiment, the kit comprises a substrate having oligonucleotides attached thereto which have a sequence complementary to the sequence of a first portion of a nucleic acid. The kit also includes a first

5   container holding liposomes having oligonucleotides attached thereto which have a sequence complementary to the sequence of a second portion of the nucleic acid and a second container holding nanoparticles having at least a first type of oligonucleotides attached thereto, the

10   first type of oligonucleotides having a cholesteryl group attached to the end not attached to the nanoparticles so that the nanoparticles can attach to the liposomes by hydrophobic interactions. The kit may further·comprise a third container holding a second type of nanoparticles

15   having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to at least a portion of the sequence of a second type of oligonucleotides attached to the first type of nanoparticles. The second type of oligonucleotides

20   attached to the first type of nanoparticles have a sequence complementary to the sequence of the oligonucleotides on the second type of nanoparticles.

In a further embodiment, the kit may comprise a first container holding nanoparticles having

25   oligonucleotides attached thereto. The kit also includes one or more additional containers, each container holding a binding oligonucleotide. Each binding oligonucleotide has a first portion which has a sequence complementary to at least a portion of the sequence of oligonucleotides on

30   the nanoparticles and a second portion which has a sequence complementary to the sequence of a portion of a nucleic acid to be detected. The sequences of the second portions of the binding oligonucleotides may be different as long as each sequence is complementary to a portion of

35   the sequence of the nucleic acid to be detected.

In another embodiment, the kit comprises a container holding one type of nanoparticles having oligonucleotides attached thereto and one or more types of binding oligonucleotides. Each of the types of binding
5   oligonucleotides has a sequence comprising at least two portions. The first portion is complementary to the sequence of the oligonucleotides on the nanoparticles, whereby the binding oligonucleotides are hybridized to the oligonucleotides on the nanoparticles in the
10   container(s). The second portion is complementary to the sequence of a portion of the nucleic acid.

In another embodiment, kits may comprise one or two containers holding two types of particles. The first type of particles having oligonucleotides attached
15   thereto which have a sequence complementary to the sequence of a first portion of a nucleic acid. The oligonucleotides are labeled with an energy donor on the ends not attached to the particles. The second type of particles having oligonucleotides attached thereto which
20   have a sequence complementary to the sequence of a second portion of a nucleic acid. The oligonucleotides are labeled with an energy acceptor on the ends not attached to the particles. The energy donors and acceptors may be fluorescent molecules.

25   The kits may also contain other reagents and items useful for detecting nucleic acid. The reagents may include PCR reagents, hybridization reagents, buffers, etc. Other items which may be provided as part of the kit include a solid surface (for visualizing
30   hybridization) such as a TLC silica plate, syringes, pipettes, cuvettes, containers, and a thermocycler (for controlling hybridization and de-hybridization temperatures). Reagents for functionalizing the nucleotides or nanoparticles may also be included in the
35   kit.

The precipitation of aggregated nanoparticles provides a means of separating a selected nucleic acid from other nucleic acids. This separation may be used as a step in the purification of the nucleic acid.

5   Hybridization conditions are those described above for detecting a nucleic acid. If the temperature is below the Tm (the temperature at which one-half of an oligonuceotide is bound to its complementary strand) for the binding of the oligonucleotides on the nanoparticles

10   to the nucleic acid, then sufficient time is needed for the aggregate to settle. The temperature of hybridization (e.g., as measured by Tm) varies with the type of salt (NaCl or $MgCl_2$) and its concentration. Salt compositions and concentrations are selected to promote

15   hybridization of the oligonucleotides on the nanoparticles to the nucleic acid at convenient working temperatures without inducing aggregation of the colloids in the absence of the nucleic acid.

The invention also provides a method of

20   nanofabrication. The method comprises providing at least one type of linking oligonucleotide having a selected sequence. A linking oligonucleotide used for nanofabrication may have any desired sequence and may be single-stranded or double-stranded. It may also contain

25   chemical modifications in the base, sugar, or backbone sections. The sequences chosen for the linking oligonucleotides and their lengths and strandedness will contribute to the rigidity or flexibility of the resulting nanomaterial or nanostructure, or a portion of

30   the nanomaterial or nanostructure. The use of a single type of linking oligonucleotide, as well as mixtures of two or more different types of linking oligonucleotides, is contemplated. The number of different linking oligonucleotides used and their lengths will contribute

to the shapes, pore sizes and other structural features of the resulting nanomaterials and nanostructures.

The sequence of a linking oligonucleotide will have at least a first portion and a second portion for binding

5   to oligonucleotides on nanoparticles. The first, second or more binding portions of the linking oligonucleotide may have the same or different sequences.

If all of the binding portions of a linking oligonucleotide have the same sequence, only a single

10   type of nanoparticle with oligonucleotides having a complementary sequence attached thereto need be used to form a nanomaterial or nanostructure. If the two or more binding portions of a linking oligonucleotide have different sequences, then two or more nanoparticle-

15   oligonucleotide conjugates must be used. *See, e.g.,* Figure 17. The oligonucleotides on each of the nanoparticles will have a sequence complementary to one of the two or more binding portions of the sequence of the linking oligonucleotide The number, sequence(s) and

20   length(s) of the binding portions and the distance(s), if any, between them will contribute to the structural and physical properties of the resulting nanomaterials and nanostructures. Of course, if the linking oligonucleotide comprises two or more portions, the

25   sequences of the binding portions must be chosen so that they are not complementary to each other to avoid having one portion of the linking nucleotide bind to another portion.

The linking oligonucleotides and nanoparticle-

30   oligonucleotide conjugates are contacted under conditions effective for hybridization of the oligonucleotides attached to the nanoparticles with the linking oligonucleotides so that a desired nanomaterial or nanostructure is formed wherein the nanoparticles are

35   held together by oligonucleotide connectors. These

hybridization conditions are well known in the art and can be optimized for a particular nanofabrication scheme (see above). Stringent hybridization conditions are preferred.

5   The invention also provides another method of nanofabrication. This method comprises providing at least two types of nanoparticle-oligonucleotide conjugates. The oligonucleotides on the first type of nanoparticles have a sequence complementary to that of

10   the oligonucleotides on the second type of nanoparticles. The oligonucleotides on the second type of nanoparticles have a sequence complementary to that of the oligonucleotides on the first type of nanoparticles. The nanoparticle-oligonucleotide conjugates are contacted

15   under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles to each other so that a desired nanomaterial or nanostructure is formed wherein the nanoparticles are held together by oligonucleotide connectors. Again, these hybridization

20   conditions are well-known in the art and can be optimized for a particular nanofabrication scheme.

In both nanofabrication methods of the invention, the use of nanoparticles having one or more different types of oligonucleotides attached thereto is

25   contemplated. The number of different oligonucleotides attached to a nanoparticle and the lengths and sequences of the one or more oligonucleotides will contribute to the rigidity and structural features of the resulting nanomaterials and nanostructures.

30   Also, the size, shape and chemical composition of the nanoparticles will contribute to the properties of the resulting nanomaterials and nanostructures. These properties include optical properties, optoelectronic properties, stability in various solutions, pore and

35   channel size variation, ability to separate bioactive

molecules while acting as a filter, etc. The use of
mixtures of nanoparticles having different sizes, shapes
and/or chemical compositions, as well as the use of
nanoparticles having uniform sizes, shapes and chemical
5   composition, are contemplated.

In either fabrication method, the nanoparticles in
the resulting nanomaterial or nanostructure are held
together by oligonucleotide connectors. The sequences,
lengths, and strandedness of the oligonucleotide
10   connectors, and the number of different oligonucleotide
connectors present will contribute to the rigidty and
structural properties of the nanomaterial or
nanostructure. If an oligonucleotide connector is
partially double-stranded, its rigidity can be increased
15   by the use of a filler oligonucleotide as described above
in connection with the method of detecting nucleic acid.
The rigidity of a completely double-stranded
oligonucleotide connector can be increased by the use of
one or more reinforcing oligonucleotides having
20   complementary sequences so that they bind to the double-
stranded oligonucleotide connector to form triple-
stranded oligonucleotide connectors. The use of
quadruple-stranded oligonucleotide connectors based on
deoxyquanosine or deoxycytidine quartets is also
25   contemplated.

Several of a variety of systems for organizing
nanoparticles based on oligonucleotide hybridization are
illustrated in the figures. In a simple system (Figure
1) one set of nanoparticles bears oligonucleotides with a
30   defined sequence and another set of nonoparticles bears
oligonucleotides with a complementary sequence. On
mixing the two sets of nanoparticle-oligonucleotide
conjugates under hybridization conditions, the two types
of particles are linked by double stranded

oligonucleotide connectors which serve as spacers to
position the nanoparticles at selected distances.

An attractive system for spacing nanoparticles
involves the addition of one free linking oligonucleotide
5   as illustrated in Figure 2. The sequence of the linking
oligonucleotide will have at least a first portion and a
second portion for binding to oligonucleotides on
nanoparticles. This system is basically the same as
utilized in the nucleic acid detection method, except
10   that the length of the added linking oligonucleotide can
be selected to be equal to the combined lengths of
oligonucleotides attached to the nanoparticles. The
related system illustrated in Figure 3 provides a
convenient means to tailor the distance between
15   . nanoparticles without having to change the sets of
nanoparticle-oligonucleotide conjugates employed.

A further elaboration of the scheme for creating
defined spaces between nanoparticles is illustrated in
Figure 4. In this case a double stranded segment of DNA
20   or RNA containing overhanging ends is employed as the
linking oligonucleotide. Hybridization of the single-
stranded, overhanging segments of the linking
oligonucleotide with the oligonucleotides attached to the
nanoparticles affords multiple double-stranded
25   oligonucleotide cross-links between the nanoparticles.

Stiffer nanomaterials and nanostructures, or
portions thereof, can be generated by employing triple-
stranded oligonucleotide connectors between
nanoparticles. In forming the triple strand, one may
30   exploit either the pyrimidine:purine:pyrimidine motif
(Moser, H.E. and Dervan, P.B. *Science*, **238**, 645-650
(1987) or the purine:purine:pyrimidine motif (Pilch, D.S.
et al. *Biochemistry*, **30**, 6081-6087 (1991). An example of
the organization of nanoparticles by generating triple-
35   stranded connectors by the pyrimidine:purine:pyrimidine

54

motif are illustrated in Figure 10. In the system shown
in Figure 10, one set of nanoparticles is conjugated with
a defined strand containing pyrimidine nucleosides and
the other set is conjugated with a complementary
5   oligonucleotide containing purine nucleosides.
Attachment of the oligonucleotides is designed such that
the nanoparticles are separated by the double-stranded
oligonucleotide formed on hybridization. Then, a free
pyrimidine oligonucleotide with an orientation opposite
10   that for the pyrimidine strand linked to the nanoparticle
is added to the system prior to, simultaneously with, or
just subsequent to mixing the nanoparticles. Since the
third strand in this system is held by Hoogsteen base
pairing, the triple strand is relatively unstable
15   thermally. Covalent bridges spanning the breadth of the
duplex are known to stabilize triple-stranded complexes
(Salunke, M., Wu, T., Letsinger, R.L., *J. Am, Chem. Soc.*
114, 8768-8772, (1992). Letsinger, R.L. and Wu, T. *J. Am
Chem. Soc.*, **117**, 7323-7328 (1995). Prakash, G. and Kool,
20   *J. Am. Chem. Soc.*, **114**, 3523-3527 (1992).

For construction of nanomaterials and
nanostructures, it may be desirable in some cases to
"lock" the assembly in place by covalent cross-links
after formation of the nanomaterial or nanostructure by
25   hybridization of the oligonucleotide components. This
can be accomplished by incorporating functional groups
that undergo a triggered irreversible reaction into the
oligonucleotides. An example of a functional group for
this purpose is a stilbenedicarboxamide group. It has
30   been demonstrated that two stilbenedicarboxamide groups
aligned within hybridized oligonucleotides readily
undergo cross-linking on irradiation with ultraviolet
light (340 nm) (Lewis, F.D. et al. (1995) *J Am. Chem.
Soc.* **117**, 8785-8792).

Alternatively, one could employ the displacement of
a 5'-O-tosyl group from an oligonucleotide, held at the
3'-position to a nanoparticle by a mercaptoalkly group,
with a thiophosphoryl group at the 3'-end of an
5   oligonucleotide held to an nanoparticle by a
mercaptoalkyl group. In the presence of an
oligonucleotide that hybridizes to both oligonucleotides
and, thereby, brings the thiophosphoryl group into
proximity of the tosyl group, the tosyl group will be
10 displaced by the thiophosphoryl group, generating an
oligonucleotide linked at the ends to two different
nanoparticles. For displacement reactions of this type,
see Herrlein et al., *J. Am. Chem. Soc.*, **177**, 10151-10152
(1995). The fact that thiophosphoryl oligonucleotides do
15 not react with gold nanoparticles under the conditions
employed in attaching mercaptoalkyl-oligonucleotides to
gold nanoparticles enables one to prepare gold
nanoparticle-oligonucleotide conjugates anchored through
the mercapto group to the nanoparticles and containing a
20 terminal thiophosphoryl group free for the coupling
reaction.

A related coupling reaction to lock the assembled
nanoparticle system in place utilizes displacement of
bromide from a terminal bromoacetylaminonucleoside by a
25 terminal thiophosphoryl-oligonucleotide as described in
Gryaznov and Letsinger, *J. Am. Chem. Soc.*, **115**, 3808.
This reaction proceeds much like the displacement of
tosylate described above, except that the reaction is
faster. Nanoparticles bearing oligonucleotides
30 terminated with thiophosphoryl groups are prepared as
described above. For preparation of nanoparticles
bearing oligonucleotides terminated with bromoacetylamino
groups, one first prepares an oligonucleotide terminated
at one end by an aminonucleoside (*e.g.*, either 5'-amino-
35 5'-deoxythymidine or 3'-amino-3'-deoxythymidine) and at

the other end by a mercaptoalkyl group. Molecules of
this oligoncleotide are then anchored to the
nanoparticles through the mercapto groups, and the
nanoparticle-oligonucleotide conjugate is then converted

5   the N-bromoacetylamino derivative by reaction with a
bromoacetyl acylating agent.

A fourth coupling scheme to lock the assemblies in
place utilizes oxidation of nanoparticles bearing
oligonucleotides terminated by thiophosphoryl groups.

10   Mild oxidizing agents, such as potassium triiodide,
potassium ferricyanide (see Gryaznov and Letsinger,
Nucleic Acids Research, **21**, 1403) or oxygen, are
preferred.

In addition, the properties of the nanomaterials and

15   nanostructures can be altered by incorporating into the
interconnecting oligonucleotide chains organic and
inorganic functions that are held in place by covalent
attachment to the oligonucleotide chains. A wide variety
of backbone, base and sugar modifications are well known

20   (see for example Uhlmann, E., and Peyman, A. *Chemical
Reviews*, **90**, 544-584 (1990). Also, the oligonucleotide
chains could be replaced by "Peptide Nucleic Acid" chains
(PNA), in which the nucleotide bases are held by a
polypeptide backbone (see Wittung, P. et al., *Nature*,

25   **368**, 561-563 (1994).

As can be seen from the foregoing, the
nanofabrication method of the invention is extremely
versatile. By varying the length, sequence and
strandedness of the linking oligonucleotides, the number,

30   length, and sequence of the binding portions of the
linking oligonucleotides, the length, sequence and number
of the oligonucleotides attached to the nanoparticles,
the size, shape and chemical composition of the
nanoparticles, the number and types of different linking

35   oligonucleotides and nanoparticles used, and the

strandedness of the oligonucleotide connectors, nanomaterials and nanostructures having a wide range of structures and properties can be prepared. These structures and properties can be varied further by cross-

5   linking of the oligonucleotide connectors, by functionalizing the oligonucleotides, by backbone, base or sugar modifications of the oligonucleotides, or by the use of peptide-nucleic acids.

The nanomaterials and nanostructures that can be

10   made by the nanofabrication method of the invention include nanoscale mechanical devices, separation membranes, bio-filters, and biochips. It is contemplated that the nanomaterials and nanostructures of the invention can be used as chemical sensors, in computers,

15   for drug delivery, for protein engineering, and as templates for biosynthesis/nanostructure fabrication/directed assembly of other structures. See generally Seeman et al., *New J. Chem.*, **17**, 739 (1993) for other possible applications.

20   It is to be noted that the term "a" or "an" entity refers to one or more of that entity. For example, "a characteristic" refers to one or more characteristics or at least one characteristic. As such, the terms "a" (or "an"), "one or more" and "at least one" are used

25   interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" have been used interchangeably.

58

EXAMPLES

Example 1:   Preparation of Oligonucleotide-
                <u>Modified Gold Nanoparticles</u>

· A.   <u>Preparation Of Gold Nanoparticles</u>

Gold colloids (13 nm diameter) were prepared by
reduction of $HAuCl_4$ with citrate as described in Frens,
Nature Phys. Sci., **241**, 20 (1973) and Grabar, *Anal.
Chem.*, **67**, 735 (1995). Briefly, all glassware was
cleaned in aqua regia (3 parts HCl, 1 part HNO_3), rinsed
with Nanopure H_2O, then oven dried prior to use. $HAuCl_4$
and sodium citrate were purchased from Aldrich Chemical
Company. Aqueous $HAuCl_4$ (1 mM, 500 mL) was brought to
reflux while stirring. Then, 38.8 mM sodium citrate (50
mL) was added quickly. The solution color changed from
pale yellow to burgundy, and refluxing was continued for
15 min. After cooling to room temperature, the red
solution was filtered through a Micron Separations Inc. 1
micron filter. Au colloids were characterized by UV-vis
spectroscopy using a Hewlett Packard 8452A diode array
spectrophotometer and by Transmission Electron Microscopy
(TEM) using a Hitachi 8100 transmission electron
microscope. Gold particles with diameters of 13 nm will
produce a visible color change when aggregated with
target and probe oligonucleotide sequences in the 10-35
nucleotide range.

B.   <u>Synthesis Of Oligonucleotides</u>

Oligonucleotides were synthesized on a 1 micromole
scale using a Milligene Expedite DNA synthesizer in
single column mode using phosphoramidite chemistry.
Eckstein, F. (ed.) *Oligonucleotides and Analogues: A
Practical Approach* (IRL Press, Oxford, 1991). All
solutions were purchased from Milligene (DNA synthesis

grade). Average coupling efficiency varied from 98 to
99.8%, and the final dimethoxytrityl (DMT) protecting
group was not cleaved from the oligonucleotides to aid in
purification.

5   For 3'-thiol-oligonucleotides, Thiol-Modifier C3 S-S
CPG support was purchased from Glen Research and used in
the automated synthesizer. During normal cleavage from
the solid support (16 hr at 55° C), 0.05 M dithiothreitol
(DTT) was added to the NH_4OH solution to reduce the 3'
10   disulfide to the thiol. Before purification by reverse
phase high pressure liquid chromatography (HPLC), excess
DTT was removed by extraction with ethyl acetate.

        For 5'-thiol oligonucleotides, 5'-Thiol-Modifier C_6-
phosphoramidite reagent was purchased from Glen Research,
15   44901 Falcon Place, Sterling, Va 20166. The
oligonucleotides were synthesized, and the final DMT
protecting group removed. Then, 1 ml of dry acetonitrile
was added to 100 μmole of the 5' Thiol Modifier C_6-
phosphoramidite. 200 μL of the amidite solution and 200
20   μL of activator (fresh from synthesizer) were mixed and
introduced onto the column containing the synthesized
oligonucleotides still on the solid support by syringe
and pumped back and forth through the column for 10
minutes. The support was then washed (2 x 1 mL) with dry
25   acetonitrile for 30 seconds. 700 μL of a 0.016 M
I_2/H_2O/pyridine mixture (oxidizer solution) was introduced
into the column, and was then pumped back and forth
through the column with two syringes for 30 second. The
support was then washed with a 1:1 mixture of
30   CH_3CN/pyridine (2 x 1 mL) for 1 minute, followed by a
final wash with dry acetonitrile (2 x 1 mL) with
subsequent drying of the column with a stream of
nitrogen. The trityl protecting group was not removed,
which aids in purification.

60

Reverse phase HPLC was performed with a Dionex DX500 system equipped with a Hewlett Packard ODS hypersil column (4.6 x 200 mm, 5 mm particle size) using 0.03 M $Et_3NH^+ OAc^-$ buffer (TEAA), pH 7, with a 1%/min. gradient of 95% CH_3CN/5% TEAA. The flow rate was 1 mL/ min. with UV detection at 260 nm. Preparative HPLC was used to purify the DMT-protected unmodified oligonucleotides (elution at 27 min). After collection and evaporation of the buffer, the DMT was cleaved from the oligonucleotides by treatment with 80% acetic acid for 30 min at room temperature. The solution was then evaporated to near dryness, water was added, and the cleaved DMT was extracted from the aqueous oligonucleotide solution using ethyl acetate. The amount of oligonucleotide was determined by absorbance at 260 nm, and final purity assessed by reverse phase HPLC (elution time 14.5 minutes).

The same protocol was used for purification of the 3'-thiol-oligonucleotides, except that DTT was added after extraction of DMT to reduce the amount of disulfide formed. After six hours at 40ºC, the DTT was extracted using ethyl acetate, and the oligonucleotides repurified by HPLC (elution time 15 minutes).

For purification of the 5' thiol modified oligonucleotides, preparatory HPLC was performed under the same conditions as for unmodified oligonucleotides. After purification, the trityl protecting group was removed by adding 150 µL of a 50 mM $AgNO_3$ solution to the dry oligonucleotide sample. The sample turned a milky white color as the cleavage occurred. After 20 minutes, 200 µL of a 10 mg/ml solution of DTT was added to complex the Ag (five minute reaction time), and the sample was centrifuged to precipitate the yellow complex. The oligonucleotide solution (<50 OD) was then transferred onto a desalting NAP-5 column (Pharmacia Biotech,

Uppsala, Sweden) for purification (contains DNA Grade
Sephadex G-25 Medium for desalting and buffer exchange of
oligonucleotides greater than 10 bases). The amount of
5' thiol modified oligonucleotide was determined by UV-
5 vis spectroscopy by measuring the magnitude of the
absorbance at 260 nm. The final purity was assessed by
performing ion-exchange HPLC with a Dionex Nucleopac PA-
100 (4 x 250) column using a 10 mM NaOH solution (pH 12)
with a 2%/min gradient of 10 mM NaOH, 1M NaCl solution.
10 Typically, two peaks resulted with elution times of
approximately 19 minutes and 25 minutes (elution times
are dependent on the length of the oligonucleotide
strand). These peaks corresponded to the thiol and the
disulfide oligonucleotides respectively.
15

C. Attachment Of Oligonucleotides To Gold Nanoparticles
An aqueous solution of 17nM (150 µL) Au colloids,
prepared as described in part A above, was mixed with
3.75 µM (46 µL) 3'-thiol-TTTGCTGA, prepared as described
20 in part B and allowed to stand for 24 hours at room
temperature in 1 ml Eppendorf capped vials. A second
solution of colloids was reacted with 3.75 µM (46 µL) 3'-
thiol-TACCGTTG. Note that these oligonucleotides are
noncomplementary. Shortly before use, equal amounts of
25 each of the two nanoparticle solutions were combined.
Since the oligonucleotides are noncomplementary, no
reaction took place.

The oligonucleotide-modified nanoparticles are
stable at elevated temperatures (80°C) and high salt
30 concentrations (1M NaCl) for days and have not been
observed to undergo particle growth. Stability in high
salt concentrations is important, since such conditions
are required for the hybridization reactions that form
the basis of the methods of detection and nanofabrication
35 of the invention.

62

Example 2:   Formation Of Nanoparticle Aggregates
       A.   Preparation Of Linking Oligonucleotide
       Two (nonthiolated) oligonucleotides were synthesized
as described in part B of Example 1. They had the
5   following sequences:
          3' ATATGCGCGA TCTCAGCAAA [SEQ ID NO:1]; and
          3' GATCGCGCAT ATCAACGGTA [SEQ ID NO:2].
       Mixing of these two oligonucleotides in a 1 M NaCl,
10 mM phosphate buffered (pH 7.0) solution, resulted in
10 hybridization to form a duplex having a 12-base-pair
overlap and two 8-base-pair sticky ends. Each of the
sticky ends had a sequence which was complementary to
that of one of the oligonucleotides attached to the Au
colloids prepared in part C of Example 1.
15

       B.   Formation Of Nanoparticle Aggregates
       The linking oligonucleotides prepared in part A of
this example (0.17 μM final concentration after dilution
with NaCl) were added to the nanoparticle-oligonucleotide
20 conjugates prepared in part C of Example 1 (5.1 nM final
concentration after dilution with NaCl) at room
temperature. The solution was then diluted with aqueous
NaCl (to a final concentration of 1 M) and buffered at pH
7 with 10 mM phosphate, conditions which are suitable for
25 hybridization of the oligonucleotides. An immediate
color change from red to purple was observed, and a
precipitation reaction ensued. See Figure 6. Over the
course of several hours, the solution became clear and a
pinkish-gray precipitate settled to the bottom of the
30 reaction vessel. See Figure 6.
       To verify that this process involved both the
oligonucleotides and colloids, the precipitate was
collected and resuspended (by shaking) in 1 M aqueous
NaCl buffered at pH 7. Any of the oligonucleotides not
35 hybridized to the nanoparticles are removed in this

63

manner. Then, a temperature/time dissociation experiment
was performed by monitoring the characteristic absorbance
for the hybridized oligodeoxyribonucleotides (260 nm) and
for the aggregated colloids which is reflective of the
5   gold interparticle distance (700 nm). See Figure 7.
Changes in absorbance at 260 and 700 nm were recorded on
a Perkin-Elmer Lambda 2 UV-vis Spectrophotometer using a
Peltier PTP-1 Temperature Controlled Cell Holder while
cycling the temperature at a rate of 1°C/minute between
10   0°C and 80°C. DNA solutions were approximately 1
absorbance unit(s) (OD), buffered at pH 7 using 10 mM
phosphate buffer and at 1M NaCl concentration.

The results are shown in Figure 8A. As the
temperature was cycled between 0°C and 80°C (which is 38°C
15   above the dissociation temperature (T_m) for the duplex
(T_m=42 °C)), there was an excellent correlation between
the optical signatures for both the colloids and
oligonucleotides. The UV-vis spectrum for naked Au
colloids was much less temperature dependent, Figure 8B.
20   There was a substantial visible optical change when
the polymeric oligonucleotide-colloid precipitate was
heated above its melting point. The clear solution
turned dark red as the polymeric biomaterial de-
hybridized to generate the unlinked colloids which are
25   soluble in the aqueous solution. The process was
reversible, as evidenced by the temperature traces in
Figure 8A.

In a control experiment, a 14-T:14-A duplex was
shown to be ineffective at inducing reversible Au colloid
30   particle aggregation. In another control experiment, a
linking oligonucleotide duplex with four base pair
mismatches in the sticky ends was found not to induce
reversible particle aggregation of oligonucleotide-
modified nanoparticles (prepared as described in part C
35   of Example 1 and reacted as described above). In a third

control experiment, non-thiolated oligonucleotides having
sequences complementary to the sticky ends of the linking
oligonucleotide and reacted with nanoparticles did not
produce reversible aggregation when the nanoparticles

5   were combined with the linking oligonucleotide.

Further evidence of the polymerization/assembly
process came from Transmission Electron Microscopy (TEM)
studies of the precipitate. TEM was performed on a
Hitachi 8100 Transmission Electron Microscope. A typical

10   sample was prepared by dropping 100 μL of colloid
solution onto a holey carbon grid. The grid, then, was
dried under vacuum and imaged. TEM images of Au colloids
linked by hybridized oligonucleotides showed large
assembled networks of the Au colloids, Figure 9A. Naked

15   Au colloids do not aggregate under comparable conditions
but rather disperse or undergo particle growth reactions.
Hayat, *Colloidal Gold: Principles, Methods, and
Applications* (Academic Press, San Diego, 1991). Note
that there is no evidence of colloid particle growth in

20   the experiments performed to date; the hybridized
colloids seem to be remarkably regular in size with an
average diameter of 13 nm.

With TEM, a superposition of layers is obtained,
making it difficult to assess the degree of order for

25   three-dimensional aggregates. However, smaller scale
images of single layer, two-dimensional aggregates
provided more evidence for the self-assembly process,
Figure 9B. Close-packed assemblies of the aggregates
with uniform particle separations of approximately 60 Å

30   can be seen. This distance is somewhat shorter than the
estimated 95 Å spacing expected for colloids connected by
rigid oligonucleotide hybrids with the sequences that
were used. However, because of the nicks in the duplex
obtained after hybridization of the oligonucleotides on

35   the nanoparticles to the linking oligonucleotides, these

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were not rigid hybrids and were quite flexible. It
should be noted that this is a variable that can be
controlled by reducing the system from four overlapping
strands to three (thereby reducing the number of nicks)
5   or by using triplexes instead of duplexes.

Example 3:   Preparation of Oligonucleotide-
                Modified Gold Nanoparticles

Gold colloids (13 nm diameter) were prepared as
10   described in Example 1. Thiol-oligonucleotides
[HS$(CH_2)_6$OP(O)(O⁻)-oligonucleotide] were also prepared as
described in Example 1.

The method of attaching thiol-oligonucleotides to
gold nanoparticles described in Example 1 was found not
15   to produce satisfactory results in some cases. In
particular, when long oligonucleotides were used, the
oligonucleotide-colloid conjugates were not stable in the
presence of a large excess of high molecular weight
salmon sperm DNA used as model for the background DNA
20   that would normally be present in a diagnostic system.
Longer exposure of the colloids to the thiol-
oligonucleotides produced oligonucleotide-colloid
conjugates that were stable to salmon sperm DNA, but the
resulting conjugates failed to hybridize satisfactorily.
25   Further experimentation led to the following procedure
for attaching thiol-oligonucleotides of any length to
gold colloids so that the conjugates are stable to high
molecular weight DNA and hybridize satisfactorily.

A 1 mL solution of the gold colloids (17nM) in water
30   was mixed with excess (3.68 µM) thiol-oligonucleotide (28
bases in length) in water, and the mixture was allowed to
stand for 12-24 hours at room temperature. Then, 100 µL
of a 0.1 M sodium hydrogen phosphate buffer, pH 7.0, and
100 µL of 1.0 M NaCl were premixed and added. After 10
35   minutes, 10 µL of 1% aqueous NaN_3 were added, and the

mixture was allowed to stand for an additional 40 hours.
This "aging" step was designed to increase the surface
coverage by the thiol-oligonucleotides and to displace
oligonucleotide bases from the gold surface. Somewhat
5   cleaner, better defined red spots in subsequent assays
were obtained if the solution was frozen in a dry-ice
bath after the 40-hour incubation and then thawed at room
temperature. Either way, the solution was next
centrifuged at 14,000 rpm in an Eppendorf Centrifuge 5414
10 for about 15 minutes to give a very pale pink supernatant
containing most of the oligonucleotide (as indicated by
the absorbance at 260 nm) along with 7-10% of the
colloidal gold (as indicated by the absorbance at 520
nm), and a compact, dark, gelatinous residue at the
15 bottom of the tube. The supernatant was removed, and the
residue was resuspended in about 200 µL of buffer (10 mM
phosphate, 0.1 M NaCl) and recentrifuged. After removal
of the supernatant solution, the residue was taken up in
1.0 mL of buffer (10 mM phosphate, 0.1 M NaCl) and 10 µL
20 of a 1% aqueous solution of NaN_3. Dissolution was
assisted by drawing the solution into, and expelling it
from, a pipette several times. The resulting red master
solution was stable (i.e., remained red and did not
aggregate) on standing for months at room temperature, on
25 spotting on silica thin-layer chromatography (TLC) plates
(see Example 4), and on addition to 2 M NaCl, 10 mM $MgCl_2$,
or solutions containing high concentrations of salmon
sperm DNA.

30   Example 4:   Acceleration Of Hybridization Of
                        Nanoparticle-Oligonucleotide
                        Conjugates
        The oligonucleotide-gold colloid conjugates I and II
illustrated in Figure 11 were prepared as described in
35 Example 3. The hybridization of these two conjugates was
extremely slow. In particular, mixing samples of

conjugates I and II in aqueous 0.1 M NaCl or in 10 mM MgCl$_2$ plus 0.1 M NaCl and allowing the mixture to stand at room temperature for a day produced little or no color change.

5   Two ways were found to improve hybridization. First, faster results were obtained by freezing the mixture of conjugates I and II (each 15 nM contained in a solution of 0.1 M NaCl) in a dry ice-isopropyl alcohol bath for 5 minutes and then thawing the mixture at room
10   temperature. The thawed solution exhibited a bluish color. When 1 μL of the solution was spotted on a standard C-18 TLC silica plate (Alltech Associates), a strong blue color was seen immediately. The hybridization and consequent color change caused by the
15   freeze-thawing procedure were reversible. On heating the hybridized solution to 80°C, the solution turned red and produced a pink spot on a TLC plate. Subsequent freezing and thawing returned the system to the (blue) hybridized state (both solution and spot on a C-18 TLC plate). In a
20   similar experiment in which the solution was not refrozen, the spot obtained on the C-18 TLC plate was pink.

A second way to obtain faster results is to warm the conjugates and target. For instance, in another
25   experiment, oligonucleotide-gold colloid conjugates and an oligonucleotide target sequence in a 0.1 M NaCl solution were warmed rapidly to 65°C and allowed to cool to room temperature over a period of 20 minutes. On spotting on a C-18 silica plate and drying, a blue spot
30   indicative of hybridization was obtained. In contrast, incubation of the conjugates and target at room temperature for an hour in 0.1 M NaCl solution did not produce a blue color indicative of hybridization. Hybridization is more rapid in 0.3 M NaCl.

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68

Example 5:   Assays Using Nanoparticle-
                  Oligonucleotide Conjugates

The oligonucleotide-gold colloid conjugates 1 and 2
illustrated in Figure 12 were prepared as described in
5   Example 3, and the oligonucleotide target 3 illustrated
in Figure 12 was prepared as described in Example 2.
Mismatched and deletion targets 4, 5, 6, and 7 were
purchased from the Northwestern University Biotechnology
Facility, Chicago, IL. These oligonucleotides were
10   synthesized on a 40 nmol scale and purified on an reverse
phase C18 cartridge (OPC). Their purity was determined
by performing ion exchange HPLC.

Selective hybridization was achieved by heating
rapidly and then cooling rapidly to the stringent
15   temperature. For example, hybridization was carried out
in 100 μL of 0.1 M NaCl plus 5 mM $MgCl_2$ containing 15 nM
of each oligonucleotide-colloid conjugate 1 and 2, and 3
nanomoles of target oligonucleotide 3, 4, 5, 6, or 7,
heating to 74°C, cooling to the temperatures indicated in
20   Table 1 below, and incubating the mixture at this
temperature for 10 minutes. A 3 μL sample of each
reaction mixture was then spotted on a C-18 TLC silica
plate. On drying (5 minutes), a strong blue color
appeared if hybridization had taken place.

25   The results are presented in Table 1 below. Pink
spots signify a negative test (i.e., that the
nanoparticles were not brought together by
hybridization), and blue spots signify a positive test
(i.e., that the nanoparticles were brought into proximity
30   due to hybridization involving both of the
oligonucleotide-colloid conjugates).

TABLE 1

REACTANTS	RESULTS (COLOR)			
	45°C	50°C	60°C	74°C
1 + 2	PINK	PINK	PINK	PINK
1 + 2 + 3 (match)	BLUE	BLUE	BLUE	BLUE
1 + 2 + 4 (half complement mismatch)	PINK	PINK	PINK	PINK
1 + 2 + 5 (-6 bp)	BLUE	PINK	PINK	PINK
1 + 2 + 6 (1 bp mismatch)	BLUE	BLUE	PINK	PINK
1 + 2 + 7 (2 bp mismatch)	PINK	PINK	PINK	PINK

As can be seen in Table 1, hybridization at 60°C gave a blue spot only for the fully-matched target 3. Hybridization at 50°C yielded blue spots with both targets 3 and 6. Hybridization at 45°C gave blue spots with targets 3, 5 and 6.

In a related series, a target containing a single mismatch T nucleotide was found to give a positive test at 58°C (blue color) and a negative test (red color) at 64°C with conjugates 1 and 2. Under the same conditions, the fully-matched target (3) gave a positive test at both temperatures, showing that the test can discriminate between a target that is fully matched and one containing a single mismatched base.

Similar results were achieved using a different hybridization method. In particular, selective

70

hybridization was achieved by freezing, thawing and then
warming rapidly to the stringent temperature. For
example, hybridization was carried out in 100 µL of 0.1 M
NaCl containing 15 nM of each oligonucleotide-colloid

5　conjugate 1 and 2, and 10 picomoles of target
oligonucleotide 3, 4, 5, 6, or 7, freezing in a dry ice-
isopropyl alcohol bath for 5 minutes,' thawing at room
temperature, then warming rapidly to the temperatures
indicated in Table 2 below, and incubating the mixture at

10　this temperature for 10 minutes. A 3 µL sample of each
reaction mixture was then spotted on a C-18 TLC silica
plate. The results are presented in Table 2.

Table 2

15

Reactants (probes) + target	Results (color)				
	RT	35°C	40°C	54°C	64°C
(1 + 2) + 3	blue	blue	blue	blue	pink
(1 + 2)	pink	pink	pink	pink	pink
(1 + 2) + 4	pink	pink	pink	pink	pink
(1 + 2) + 5	blue	blue	pink	pink	pink
(1 + 2) + 6	blue	blue	blue	pink	pink
(1 + 2) + 7	blue	pink	pink	pink	pink

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An important feature of these systems was that the
color change associated with the temperature change was
30　very sharp, occurring over a temperature range of about
1°C. This indicates high cooperativity in the melting and
association processes involving the colloid conjugates
and enables one to easily discriminate between
oligonucleotide targets containing a fully-matched
35　sequence and a single basepair mismatch.
The high degree of discrimination may be attributed
to two features. The first is the alignment of two

relatively short probe oligonucleotide segments (15 nucleotides) on the target is required for a positive signal. A mismatch in either segment is more destabilizing than a mismatch in a longer probe (e.g., an oligonucleotide 30 bases long) in a comparable two-component detection system. Second, the signal at 260 nm, obtained on hybridization of the target oligonucleotides with the nanoparticle conjugates in solution, is nanoparticle-based, not DNA-based. It depends on dissociation of an assembly of nanoparticles organized in a polymeric network by multiple oligonucleotide duplexes. This results in a narrowing of the temperature range that is observed for aggregate dissociation, as compared with standard DNA thermal denaturation. In short, some duplexes in the crosslinked aggregates can dissociate without dispersing the nanoparticles into solution. Therefore, the temperature range for aggregate melting is very narrow (4°C) as compared with the temperature range associated with melting the comparable system without nanoparticles (12°C). Even more striking and advantageous for this detection approach is the temperature range for the colorimetric response (<1°C) observe on the C18 silica plates. In principle, this three-component nanoparticle based strategy will be more selective than any two-component detection system based on a single-strand probe hybridizing with target nucleic acid.

A master solution containing 1 nmol of target 3 was prepared in 100 μl of hybridization buffer (0.3 M NaCl, 10 mM phosphate, pH 7). One μl of this solution corresponds to 10 picomole of target oligonucleotide. Serial dilutions were performed by taking an aliquot of the master solution and diluting it to the desired concentration with hybridization buffer. Table 3 shows the sensitivity obtained using 3 μl of a mixutre of

72

probes 1 and 2 with different amounts of target 3. After
performing the hybridization using freeze-thaw
conditions, 3 µl aliquots of these solutions were spotted
onto C-18 TLC plates to determine color. In Table 3
5   below, pink signifies a negative test, and blue signifies
a positive test.

Table 3

Amount of Target	Results
1 picomole	blue (positive)
200 femtomole	blue (positive)
100 femtomole	blue (positive)
20 femtomole	blue (positive)
10 femtomole	purplish (ambiguous)

This experiment indicates that 10 femtomoles is the lower
limit of detection for this particular system.

20   Example 6:   Assays. Using Nanoparticle-
                         Oligonucleotide Conjugates
          DNA modified nanoparticles were adsorbed onto
modified transparent substrates as shown in Figure 13B,
panels 1-6. This method involved the linking of DNA
25   modified nanoparticles to nanoparticles that were
attached to a glass substrate, using DNA hybridization
interactions.

          Glass microscope slides were purchased from Fisher
scientific. Slides were cut into approximately 5 x 15 mm
30   pieces, using a diamond tipped scribing pen. Slides were
cleaned by soaking for 20 minutes in a solution of 4:1
$H_2SO_4:H_2O_2$ at 50°C. Slides were then rinsed with copious
amounts of water, then ethanol, and dried under a stream
of dry nitrogen. To functionalize the slide surface with
35   a thiol terminated silane, the slides were soaked in a
degassed ethanolic 1% (by volume)

mercaptopropyltrimethoxysilane solution for 12 hours.
The slides were removed from the ethanol solutions and
rinsed with ethanol, then water. Nanoparticles were
adsorbed onto the thiol terminated surface of the slides
5   by soaking in solutions containing the 13 nm diameter
gold nanoparticles (preparation described in Example 1).
After 12 hours in the colloidal solutions, the slides
were removed and rinsed with water. The resulting slides
have a pink appearance due to the adsorbed nanoparticles
10   and exhibit similar UV-vis absorbance profiles (surface
plasmon absorbance peak at 520 nm) as the aqueous gold
nanoparticle colloidal solutions. See Figure 14A.

       DNA was attached to the nanoparticle modified
surface by soaking the glass slides in 0.2 OD (1.7 μM)
15   solution containing freshly purified 3' thiol
oligonucleotide (3' thiol ATGCTCAACTCT [SEQ ID NO:33])
(synthesized as described in Examples 1 and 3). After 12
hours of soaking time, the slides were removed and rinsed
with water.

20   To demonstrate the ability of an analyte DNA strand
to bind nanoparticles to the modified substrate, a
linking oligonucleotide was prepared. The linking
oligonucleotide (prepared as described in Example 2) was
24 bp long (5' TACGAGTTGAGAATCCTGAATGCG [SEQ ID NO:34])
25   with a sequence containing a 12 bp end that was
complementary to the DNA already adsorbed onto the
substrate surface (SEQ ID NO:33). The substrate was then
soaked in a hybridization buffer (0.5 M NaCl, 10 mM
phosphate buffer pH 7) solution containing the linking
30   oligonucleotide (0.4 OD, 1.7 μM) for 12 hours. After
removal and rinsing with similar buffer, the substrate
was soaked in a solution containing 13 nm diameter gold
nanoparticles which had been modified with an
oligonucleotide (TAGGACTTACGC 5' thiol [SEQ ID NO:35])
35   (prepared as described in Example 3) that is

74

complementary to the unhybridized portion of the linking
oligonucleotide attached to the substrate. After 12
hours of soaking, the substrate was removed and rinsed
with the hybridization buffer. The substrate color had

5   darkened to a purple color and the UV-vis absorbance at
520 nm approximately doubled (Figure 14A).

To verify that the oligonucleotide modified gold
nanoparticles were attached to the oligonucleotide/
nanoparticle modified surface through DNA hybridization

10   interactions with the linking oligonucleotide, a melting
curve was performed. For the melting experiment, the
substrate was placed in a cuvette containing 1 mL of
hybridization buffer and the same apparatus used in
Example 2, part B, was used. The absorbance signal due

15   to the nanoparticles (520 nm) was monitored as the
temperature of the substrate was increased at a rate of
0.5°C per minute. The nanoparticle signal dramatically
dropped when the temperature passed 60°C. See Figure
14B. A first derivative of the signal showed a melting

20   temperature of 62°C, which corresponds with the
temperature seen for the three DNA sequences hybridized
in solution without nanoparticles. See Figure 14B.

25   Example 7:   Assays Using Nanoparticle-
                         Oligonucleotide Conjugates

The detection system illustrated in Figure 15 was
designed so that the two probes 1 and 2 align in a tail-
to-tail fashion onto a complementary target 4 (see Figure
15). This differs from the system described in Example 5

30   where the two probes align contiguously on the target
strand (see Figure 12).

The oligonucleotide-gold nanoparticle conjugates 1
and 2 illustrated in Figure 15 were prepared as described
in Example 3, except that the nanoparticles were

35   redispersed in hybridization buffer (0.3 M NaCl, 10 mM

phosphate, pH 7). The final nanoparticle-oligonucleotide conjugate concentration was estimated to be 13 nM by measuring the reduction in intensity of the surface plasmon band at 522 nm which gives rise to the red color

5   of the nanoparticles. The oligonucleotide targets illustrated in Figure 15 were purchased from the Northwestern University Biotechnology Facility, Evanston, IL.

       When 150 μL of hybridization buffer containing 13 nM

10   oligonucleotide-nanoparticle conjugates 1 and 2 was mixed with 60 picomoles (6 μL) of target 4, the solution color immediately changed from red to purple. This color change occurs as·a result of the formation of large oligonucleotide-linked polymeric networks of gold

15   nanoparticles, which leads to a red shift in the surface plasmon resonance of the nanoparticles. When the solution was allowed to stand for over 2 hours, precipitation of large macroscopic aggregates was observed. A 'melting analysis' of the solution with the

20   suspended aggregates was performed. To perform the 'melting analysis', the solution was diluted to 1 ml with hybridization buffer, and the optical signature of the aggregates at 260 nm was recorded at one minute intervals as the temperature was increased from 25°C to 75°C, with a

25   holding time of 1 minute/degree. Consistent with characterization of the aggregate as an oligonucleotide-nanoparticle polymer, a characteristic sharp transition (full width at half maximum, $FW_{1/2}$ of the first derivative = 3.5°C) was observed with a "melting temperature" (T_m)

30   of 53.5°C. This compares well with the T_m associated with the broader transition observed for oligonucleotides without nanoparticles (T_m = 54°C, $FW_{1/2}$ = ~13.5°C). The 'melting analysis' of the oligonucleotide solution without nanoparticles was performed under similar

35   conditions as the analysis with nanoparticles, except

that the temperature was increased from 10-80 °C. Also,
the solution was 1.04 μM in each oligonucleotide
component.

To test the selectivity of the system, the T_m for the
aggregate formed from the perfect complement 4 of probes
1 and 2 was compared with the T_m's for aggregates formed
from targets that contained one base mismatches,
deletions, or insertions (Figure 15).
Significantly, all of the gold nanoparticle-
oligonucleotide aggregates that contained imperfect
targets exhibited significant, measurable destabilization
when compared to the aggregates formed from the perfect
complement, as evidenced by T_m values for the various
aggregates (see Figure 15). The solutions containing the
imperfect targets could easily be distinguished from the
solution containing the perfect complement by their color
when placed in a water bath held at 52.5°C. This
temperature is above the T_m of the mismatched
polynucleotides, so only the solution with the perfect
target exhibited a purple color at this temperature. A
'melting analysis' was also performed on the probe
solution which contained the half-complementary target.
Only a minute increase in absorbance at 260 nm was
observed.

Next, 2 μL (20 picomoles) of each of the
oligonucleotide targets (Figure 15) were added to a
solution containing 50 μL of each probe (13 nM) in
hybridization buffer. After standing for 15 minutes at
room temperature, the solutions were transferred to a
temperature-controlled water bath and incubated at the
temperatures indicated in Table 4 below for five minutes.
A 3 μl sample of each reaction mixture was then spotted
on a C-18 silica plate. Two control experiments were
performed to demonstrate that the alignment of both
probes onto the target is necessary to trigger

aggregation and, therefore, a color change. The first
control experiment consisted of both probes 1 and 2
without target present. The second control experiment
consisted of both probes 1 and 2 with a target 3 that is
5 complementary to only one of the probe sequences (Figure
15). The results are presented in Table 4 below. Pink
spots signify a negative test, and blue spots signify a
positive test.

Notably, the colorimetric transition that can be
10 detected by the naked eye occurs over less than $1^{\circ}C$,
thereby allowing one to easily distinguish the perfect
target 4 from the targets with mismatches (5 and 6), an
end deletion (7), and a one base insertion at the point·
in the target where the two oligonucleotide probes meet
15 (8) (see Table 4). Note that the colorimetric transition
T_c is close in temperature, but not identical, to T_m. In
both controls, there were no signs of particle
aggregation or instability in the solutions, as evidenced
by the pinkish red color which was observed at all
20 temperatures, and they showed negative spots (pink) in
the plate test at all temperatures (Table 4).

The observation that the one base insertion target 8
can be differentiated from the fully complementary target
4 is truly remarkable given the complete complementarity
25 of the insertion strand with the two probe sequences.
The destabilization of the aggregate formed from 8 and
the nanoparticle probes appears to be due to the use of
two short probes and the loss of base stacking between
the two thymidine bases where the probe tails meet when
30 hybridized to the fully complementary target. A similar
effect was observed when a target containing a three base
pair insertion (CCC) was hybridized to the probes under
comparable conditions, $(T_m = 51^{\circ}C)$. In the system
described above in Example 5, targets with base
35 insertions could not be distinguished from the fully

complementary target. Therefore, the system described in this example is very favorable in terms of selectivity. This system also exhibited the same sensitivity as the system described in Example 5, which is approximately 10
5   femtomoles without amplification techniques.

The results indicate that any one base mismatch along the target strand can be detected, along with any insertions into the target strand. Importantly, the temperature range over which a color change can be
10 detected is extremely sharp, and the change occurs over a very narrow temperature range. This sharp transition indicates that there is a large degree of cooperativity in the melting process involving the large network of colloids which are linked by the target oligonucleotide
15 strands. This leads to the remarkable selectivity as shown by the data.

Table 4

Reactants (probes) + target	Results (color)					
	RT	47.6°C	50.5°C	51.4°C	52.7°C	54.5°C
(1 + 2)	pink	pink	pink	pink	pink	pink
(1 + 2) + 3	pink	pink	pink	pink	pink	pink
(1 + 2) + 4	blue	blue	blue	blue	blue	pink
(1 + 2) + 5	blue	blue	blue	pink	pink	pink
(1 + 2) + 6	blue	pink	pink	pink	pink	pink
(1 + 2) + 7	blue	blue	blue	blue	pink	pink
(1 + 2) + 8	blue	blue	pink	pink	pink	pink

Example 8:   Assays Using Nanoparticle-
               Oligonucleotide Conjugates

A set of experiments were performed involving
hybridization with 'filler' duplex oligonucleotides.
5   Nanoparticle-oligonucleotide conjugates 1 and 2
illustrated in Figure 16 were incubated with targets of
different lengths (24, 48 and 72 bases in length) and
complementary filler oligonucleotides, as illustrated in
Figure 16. Otherwise, the conditions were as described
10 in Example 7. Also, the oligonucleotides and
nanoparticle-oligonucleotide conjugates were prepared as
described in Example 7.

As expected, the different reaction solutions had
markedly different optical properties after hybridization
15 due to the distance-dependent optical properties of the
gold nanoparticles. See Table 5 below. However, when
these solutions were spotted onto a C-18 TLC plate, a
blue color developed upon drying at room temperature or
80°C, regardless of the length of the target
20 oligonucleotide and the distance between the gold
nanoparticles. See Table 5. This probably occurs
because the solid support enhances aggregation of the
hybridized oligonucleotide-nanoparticle conjugates. This
demonstrates that by spotting solutions onto the TLC
25 plate, the distance between the gold nanoparticles can be
substantial (at least 72 bases), and colorimetric
detection is still possible.

80

TABLE 5

TARGET LENGTH	RESULTS (COLOR)	
	SOLUTION	TLC PLATE
24 BASES	BLUE	BLUE
48 BASES	PINK	BLUE
72 BASES	PINK	BLUE
PROBES 1 + 2 ONLY	PINK	PINK

The color changes observed in this and other examples occur when the distance between the gold nanoparticles (the interparticle distance) is approximately the same or less than the diameter of the nanoparticle. Thus, the size of the nanoparticles, the size of the oligonucleotides attached to them, and the spacing of the nanoparticles when they are hybridized to the target nucleic acid affect whether a color change will be observable when the oligonucleotide-nanoparticle conjugates hybridize with the nucleic acid targets to form aggregates. For instance, gold nanoparticles with diameters of 13 nm will produce a color change when aggregated using oligonucleotides attached to the nanoparticles designed to hybridize with target sequences 10-35 nucleotides in length. The spacing of the nanoparticles when they are hybridized to the target nucleic acid adequate to give a color change will vary with the extent of aggregation, as the results demonstrate. The results also indicate that the solid surface enhances further aggregation of already-aggregated samples, bringing the gold nanoparticles closer together.

The color change observed with gold nanoparticles is attributable to a shift and broadening of the surface

plasmon resonance of the gold. This color change is unlikely for gold nanoparticles less than about 4 nm in diameter because the lengths of the oligonucleotides necessary for specific detection of nucleic acid would

5 exceed the nanoparticle diameter.

Example 9:   Assays Using Nanoparticle-
                Oligonucleotide Conjugates

Five microliters of each probe 1 and 2 (Figure 12)

10 were combined to a final concentration of 0.1 M NaCl with buffer (10 mM phosphate, pH 7), and 1 microliter of human urine was added to the solution. When this solution was frozen, thawed, and then spotted on a C-18 TLC plate, a blue color did not develop. To a similar solution

15 containing 12.5 microliters of each probe and 2.5 microliters of human urine, 0.25 microliters (10 picomoles) of target 3 (Figure 12) was added. The solution was frozen, thawed and then spotted onto a C-18 TLC plate, and a blue spot was obtained.

20   Similar experiments were performed in the presence of human saliva. A solution containing 12.5 microliters of each probe 1 and 2 and 0.25 microliters of target 3 was heated to 70°C. After cooling to room temperature, 2.5 microliters of a saliva solution (human saliva

25 diluted 1:10 with water) was added. After the resultant solution was frozen, thawed and then spotted onto a C-18 TLC plate, a blue spot was obtained, indicating hybridization of the probes with the target. In control experiments with no target added, blue spots were not

30 observed.

Example 10:   Assays Using Nanoparticle-
                Oligonucleotide Conjugates

An assay was performed as illustrated in Figure 13A.

35 First, glass microscope slides, purchased from Fisher scientific, were cut into approximately 5 x 15 mm pieces,

using a diamond tipped scribing pen. Slides were cleaned
by soaking for 20 minutes in a solution of 4:1 $H_2SO_4:H_2O_2$
at 50°C. Slides were then rinsed with copious amounts of
water, then ethanol, and dried under a stream of dry

5   nitrogen. Thiol-modified DNA was adsorbed onto the
slides using a modified procedure reported in the
literature (Chrisey et al., *Nucleic Acids Res.*, **24**, 3031-
3039 (1996) and Chrisey et al., *Nucleic Acids Res.*, **24**,
3040-3047 (1996)). First, the slides were soaked in a 1%

10   solution of trimethoxysilylpropyldiethyltriamine (DETA,
purchased from United Chemical Technologies, Bristol, PA)
in 1 mM acetic acid in Nanopure water for 20 minutes at
room temperature. The slides were rinsed with water,
then ethanol. After drying with a dry nitrogen stream,

15   the slides were baked at 120°C for 5 minutes using a
temperature-controlled heating block. The slides were
allowed to cool, then were soaked in a 1 mM succinimidyl
4-(malemidophenyl)-butyrate (SMPB, purchased from Sigma
Chemicals) solution in 80:20 methanol:dimethoxysulfoxide

20   for 2 hours at room temperature. After removal from the
SMPB solution and rinsing with ethanol, amine sites that
were not coupled to the SMPB crosslinker were capped as
follows. First, the slides were soaked for 5 minutes in
a 8:1 THF:pyridine solution containing 10% 1-methyl

25   imidazole. Then the slides were soaked in 9:1 THF:acetic
anhydride solution for five minutes. These capping
solutions were purchased from Glen Research, Sterling,
VA. The slides were rinsed with THF, then ethanol, and
finally water.

30   DNA was attached to the surfaces by soaking the
modified glass slides in a 0.2 OD (1.7 μM) solution
containing freshly purified oligonucleotide (3' thiol
ATGCTCAACTCT [SEQ ID NO:33]). After 12 hours of soaking
time, the slides were removed and rinsed with water.

To demonstrate the ability of an analyte DNA strand
to bind nanoparticles to the modified substrate, a
linking oligonucleotide was prepared. The linking
oligonucleotide was 24 bp long (5' TACGAGTTGA

5   GAATCCTGAATGCG [SEQ ID NO:34]) with a sequence containing
a 12 bp end that was complementary to the DNA already
adsorbed onto the substrate surface. The substrate was
then soaked in a hybridization buffer (0.5 M NaCl, 10 mM
phosphate buffer pH 7) solution containing the linking

10   oligonucleotide (0.4 OD, 1.7 µM) for 12 hours. After
removal and rinsing with similar buffer, the substrate
was soaked in a solution containing 13 nm diameter gold
nanoparticles which had been modified with an
oligonucleotide (TAGGACTTACGC 5' thiol [SEQ ID NO:35])

15   that is complementary to the unhybridized portion of the
linking oligonucleotide attached to the substrate. After
12 hours of soaking, the substrate was removed and rinsed
with the hybridization buffer. The glass substrate's
color had changed from clear and colorless to a

20   transparent pink color. See Figure 19A.
         Additional layers of nanoparticles were added to the
slides by soaking the slides in a solution of the linking
oligonucleotide as described above and then soaking in a
solution containing 13 nm gold nanoparticled having

25   oligonucleotides (3' thiol ATGCTCAACTCT [SEQ ID NO:33])
attached thereto. After soaking for 12 hours, the slides
were removed from the nanoparticle solution and rinsed
and soaked in hybridization buffer as described above.
The color of the slide had become noticeably more red.

30   See Figure 19A. A final nanoparticle layer was added by
repeating the linking oligonucleotide and nanoparticle
soaking procedures using 13 nm gold nanoparticles which
had been modified with an oligonucleotide (TAGGACTTACGC
5' thiol [SEQ ID NO:35]) as the final nanoparticle layer.

84

Again, the color darkened, and the UV-vis absorbance at
520 nm increased. See Figure 19A.

　　　To verify that the oligonucleotide modified gold
nanoparticles were attached to the oligonucleotide
5   modified surface through DNA hybridization interactions
with the linking oligonucleotide, a melting curve was
performed. For the melting experiment, a slide was
placed in a cuvette containing 1.5 mL of hybridization
buffer, and an apparatus similar to that used in Example
10 2, part B, was used. The absorbance signal due to the
nanoparticles (520 nm) was monitored at each degree as
the temperature of the substrate was increased from 20°C
to 80°C, with a hold time of 1 minute at each integral
degree. The nanoparticle signal dramatically dropped
15 when the temperature passed 52°C. See Figure 19B. A
first derivative of the signal showed a melting
temperature of 55°C, which corresponds with the
temperature seen for the oligonucleotide-nanoparticle
conjugates and linking oligonculeotides hybridized in
20 solution. See Figure 19B.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: MIRKIN, CHAD A.
                LETSINGER, ROBERT L.
                MUCIC, ROBERT C.
                STORHOFF, JAMES J.
                ELGHANIAN, ROBERT

    (ii) TITLE OF INVENTION: NANOPARTICLES HAVING OLIGONUCLEOTIDES
        ATTACHED THERETO AND USES THEREFOR

    (iii) NUMBER OF SEQUENCES: 35

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    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE:
        (C) CLASSIFICATION:

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        (C) REFERENCE/DOCKET NUMBER: 3501-14-PCT

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(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
        (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAACGACTCT AGCGCGTATA   20

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
        (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATGGCAACTA TACGCGCTAG   20

(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 16 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
        (A) DESCRIPTION: /desc = "hypothetical"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCTTGAGATT TCCCTC

16

(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 16 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
        (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GAGGGAAATC TCAAGG

16

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 18 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
        (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AACTTGCGCT AATGGCGA

18

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 26 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
        (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AAGTTGCGCT TTACGGCTAA TGGCGA   26

(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 15 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
        (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCTCCTTCCC TTTTC   15

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 15 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
        (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAAAAGGGAA GGAGA   15

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 15 base pairs
        (B) TYPE: nucleic acid

89

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        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear


    (ii) MOLECULE TYPE: other nucleic acid
        (A) DESCRIPTION: /desc = "hypothetical"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:


CTTTTCCCTT CCTCT                                                        15


(2) INFORMATION FOR SEQ ID NO:10:


    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 28 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear


    (ii) MOLECULE TYPE: other nucleic acid
        (A) DESCRIPTION: /desc = "hypothetical"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:


AAACGACTCT AGCGCGTATA GTTGCCAT                                          28


(2) INFORMATION FOR SEQ ID NO:11:


    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 28 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear


    (ii) MOLECULE TYPE: other nucleic acid
        (A) DESCRIPTION: /desc = "hypothetical"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:


ATGGCAACTA TACGCGCTAG AGTCGTTT                                          28
```

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 15 base pairs

        (B) TYPE: nucleic acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid

        (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCTATCGACC ATGCT   15

(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 30 base pairs

        (B) TYPE: nucleic acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid

        (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGCATGGTCG ATAGGAAACG ACTCTAGCGC   30

(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 15 base pairs

        (B) TYPE: nucleic acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid

        (A) DESCRIPTION: /desc = "hypothetical"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCGCTAGAGT CGTTT   15

(2) INFORMATION FOR SEQ ID NO:15:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 30 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
       (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AGCATGGTCG ATAGGATGGC AACTATACGC   30

(2) INFORMATION FOR SEQ ID NO:16:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 24 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
       (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTCGATAGGA AACGACTCTA GCGC   24

(2) INFORMATION FOR SEQ ID NO:17:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 30 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: other nucleic acid
       (A) DESCRIPTION: /desc = "hypothetical"

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AGCATGGTTG ATAGGAAACG ACTCTAGCGC   30

(2) INFORMATION FOR SEQ ID NO:18:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 30 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: other nucleic acid
        (A) DESCRIPTION: /desc = "hypothetical"

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AGCATGTTTG ATAGGAAACG ACTCTAGCGC   30

(2) INFORMATION FOR SEQ ID NO:19:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 12 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: other nucleic acid
        (A) DESCRIPTION: /desc = "hypothetical"

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCTCAACTCG TA   12

(2) INFORMATION FOR SEQ ID NO:20:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 12 base pairs

        (B) TYPE: nucleic acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid

        (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CGCATTCAGG AT   12

(2) INFORMATION FOR SEQ ID NO:21:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 24 base pairs

        (B) TYPE: nucleic acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid

        (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TACGAGTTGA GAGAGTGCCC ACAT   24

(2) INFORMATION FOR SEQ ID NO:22:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 24 base pairs

        (B) TYPE: nucleic acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid

        (A) DESCRIPTION: /desc = "hypothetical"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TACGAGTTGA GAATCCTGAA TGCG

24

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 24 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
     (A) DESCRIPTION: /desc = "hypothetical"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TACGAGTTGA GAATCCTGAA TGCT

24

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 24 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
     (A) DESCRIPTION: /desc = "hypothetical"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TACGAGTTGA GACTCCTGAA TGCG

24

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 23 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: other nucleic acid
              (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

  TACGAGTTGA GAATCCTGAA TGC   23

  (2) INFORMATION FOR SEQ ID NO:26:

        (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 25 base pairs
              (B) TYPE: nucleic acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: other nucleic acid
              (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

  TACGAGTTGA GACATCCTGA ATGCG   25

  (2) INFORMATION FOR SEQ ID NO:27:

        (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 24 base pairs
              (B) TYPE: nucleic acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: other nucleic acid
              (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

  TACGAGTTGA GAATCCTGAA TGCG   24

(2) INFORMATION FOR SEQ ID NO:28:

    (i) SEQUENCE CHARACTERISTICS:

       (A) LENGTH: 12 base pairs

       (B) TYPE: nucleic acid

       (C) STRANDEDNESS: single

       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid

       (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CGCATTCAGG AT   12

(2) INFORMATION FOR SEQ ID NO:29:

    (i) SEQUENCE CHARACTERISTICS:

       (A) LENGTH: 48 base pairs

       (B) TYPE: nucleic acid

       (C) STRANDEDNESS: single

       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid

       (A) DESCRIPTION: /desc = "hypothetical"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TACGAGTTGA GACCGTTAAG ACGAGGCAAT CATGCAATCC TGAATGCG   48

(2) INFORMATION FOR SEQ ID NO:30:

    (i) SEQUENCE CHARACTERISTICS:

       (A) LENGTH: 24 base pairs

       (B) TYPE: nucleic acid

       (C) STRANDEDNESS: single

       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid

       (A) DESCRIPTION: /desc = "hypothetical"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TGCATGATTG CCTCGTCTTA ACGG   24

(2) INFORMATION FOR SEQ ID NO:31:

      (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 72 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: other nucleic acid
         (A) DESCRIPTION: /desc = "hypothetical"

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TACGAGTTGA GACCGTTAAG ACGAGGCAAT CATGCATATA TTGGACGCTT TACGGACAAC   60

ATCCTGAATG CG   72

(2) INFORMATION FOR SEQ ID NO:32:

      (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 48 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: other nucleic acid
         (A) DESCRIPTION: /desc = "hypothetical"

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GTTGTCCGTA AAGCGTCCAA TATATGCATG ATTGCCTCGT CTTAACGG   48

(2) INFORMATION FOR SEQ ID NO:33:
      (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 12 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

98

(ii) MOLECULE TYPE: other nucleic acid
      (A) DESCRIPTION: /desc = "hypothetical"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TCTCAACTCG TA   12

(2) INFORMATION FOR SEQ ID NO:34:

      (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 24 base pairs
           (B) TYPE: nucleic acid
           (C) STRANDEDNESS: single
           (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: other nucleic acid
           (A) DESCRIPTION: /desc = "hypothetical"

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TACGAGTTGA GAATCCTGAA TGCG   24

(2) INFORMATION FOR SEQ ID NO:35:

      (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 12 base pairs
           (B) TYPE: nucleic acid
           (C) STRANDEDNESS: single
           (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: other nucleic acid
           (A) DESCRIPTION: /desc = "hypothetical"

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CGCATTCAGG AT   12

WE CLAIM:

1.   A method of detecting a nucleic acid having at least two portions comprising:

5   providing one or more types of nanoparticles having oligonucleotides attached thereto, the oligonucleotides on each of the types of nanoparticles having a sequence complementary to the sequence of one of the portions of the nucleic acid;

10   contacting the nucleic acid and the nanoparticles under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with the nucleic acid; and

           observing a detectable change brought about by
15 hybridization of the oligonucleotides on the nanoparticles with the nucleic acid.

2.   A method of detecting nucleic acid having at least two portions comprising:

20   contacting the nucleic acid with at least two types of nanoparticles having oligonucleotides attached thereto, the oligonucleotides on the first type of nanoparticles having a sequence complementary to a first portion of the sequence of the nucleic acid, the
25 oligonucleotides on the second type of nanoparticles having a sequence complementary to a second portion of the sequence of the nucleic acid, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with the
30 nucleic acid; and

           observing a detectable change brought about by hybridization of the oligonucleotides on the nanoparticles with the nucleic acid.

100

3.   The method of Claim 2 wherein the contacting conditions include freezing and thawing.

4.   The method of Claim 2 wherein the contacting
5   conditions include heating.

5.   The method of Claim 2 wherein the detectable change is observed on a solid surface.

10   6.   The method of Claim 2 wherein the detectable change is a color change observable with the naked eye.

7.   The method of Claim 6 wherein the color change is observed on a solid surface.
15

8.   The method of Claim 2 wherein the nanoparticles are made of gold.

9.   The method of Claim 2 wherein the nanoparticles
20   are metallic or semiconductor nanoparticles and the oligonucleotides attached to the nanoparticles are labeled with fluorescent molecules on the ends not attached to the nanoparticles.

25   10.   The method of Claim 2 wherein:
        the nucleic acid has a third portion located between the first and second portions, and the sequences of the oligonucleotides on the nanoparticles do not include sequences complementary to this third portion of
30   the nucleic acid; and
        the nucleic acid is further contacted with a filler oligonucleotide having a sequence complementary to this third portion of the nucleic acid, the contacting taking place under conditions effective to allow

hybridization of the filler oligonucleotide with the nucleic acid.

11. The method of Claim 2 wherein the nucleic acid is viral RNA or DNA.

12. The method of Claim 2 wherein the nucleic acid is a gene associated with a disease.

13. The method of Claim 2 wherein the nucleic acid is a bacterial DNA.

14. The method of Claim 2 wherein the nucleic acid is a fungal DNA.

15. The method of Claim 2 wherein the nucleic acid is a synthetic DNA, a synthetic RNA, a structurally-modified natural or synthetic RNA, or a structurally-modified natural or synthetic DNA.

16. The method of Claim 2 wherein the nucleic acid is from a biological source.

17. The method of Claim 2 wherein the nucleic acid is a product of a polymerase chain reaction amplification.

18. The method of Claim 2 wherein the nucleic acid is a fragment obtained by cleavage of DNA with a restriction enzyme.

19. The method of Claim 2 wherein the nucleic acid is contacted with the first and second types of nanoparticles simultaneously.

20. The method of Claim 2 wherein the nucleic acid is contacted and hybridized with the oligonucleotides on the first type of nanoparticles before being contacted with the second type of nanoparticles.

5

21. The method of Claim 20 wherein the first type of nanoparticles is attached to a substrate.

22. A method of detecting nucleic acid having at least two portions comprising:

providing a substrate having a first type of nanoparticles attached thereto, the nanoparticles having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to a first portion of the sequence of the nucleic acid;

contacting the nucleic acid with the nanoparticles attached to the substrate under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with the nucleic acid;

providing a second type of nanoparticles having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to one or more other portions of the sequence of the nucleic acid;

contacting the nucleic acid bound to the substrate with the second type of nanoparticles under conditions effective to allow hybridization of the oligonucleotides on the second type of nanoparticles with the nucleic acid; and

observing a detectable change.

23. The method of Claim 22 wherein the nanoparticles are made of gold.

24. A method of detecting nucleic acid having at least two portions comprising:

providing a substrate having a first type of nanoparticles attached thereto, the nanoparticles having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to a first portion of the

5   sequence of the nucleic acid;

contacting the nucleic acid with the nanoparticles attached to the substrate under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with the nucleic acid;

10   providing a second type of nanoparticles having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to one or more other portions of the sequence of the nucleic acid;

contacting the nucleic acid bound to the

15   substrate with the second type of nanoparticles under conditions effective to allow hybridization of the oligonucleotides on the second type of nanoparticles with the nucleic acid;

providing a binding oligonucleotide having a

20   selected sequence having at least two portions, the first portion being complementary to at least a portion of the sequence of the oligonucleotides on the second type of nanoparticles;

contacting the binding oligonucleotide with the

25   second type of nanoparticles bound to the substrate under conditions effective to allow hybridization of the binding oligonucleotide to the oligonucleotides on the nanoparticles;

providing a third type of nanoparticles having

30   oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to the sequence of a second portion of the binding oligonucleotide;

contacting the third type of nanoparticles with the binding oligonucleotide bound to the substrate under

35   conditions effective to allow hybridization of the

binding oligonucleotide to the oligonucleotides on the nanoparticles; and

observing a detectable change.

5   25.   The method of Claim 24 wherein the substrate is transparent.

       26.   The method of Claim 25 wherein the detectable change is the formation of dark areas on the substrate.
10

       27.   The method of Claim 24 wherein the nanoparticles are made of gold.

       28.   A method of detecting nucleic acid having at
15   least two portions comprising:

       contacting the nucleic acid with a substrate having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to a first portion of the sequence of the nucleic acid, the
20   contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the substrate with the nucleic acid;

       contacting the nucleic acid bound to the substrate with a first type of nanoparticles having
25   oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to one or more other portions of the sequence of the nucleic acid, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the
30   nanoparticles with the nucleic acid;

       contacting the first type of nanoparticles bound to the substrate with a second type of nanoparticles having oligonucleotides attached thereto, the oligonucleotides on the second type of nanoparticles
35   having a sequence complementary to at least a portion of

the sequence of the oligonucleotides on the first type of nanoparticles, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the first and second types of
5   nanoparticles; and

observing a detectable change.

29.   The method of Claim 28 wherein the substrate is transparent.
10

30.   The method of Claim 29 wherein the detectable change is the formation of dark areas on the substrate.

31.   The method of Claim 28 wherein the
15   nanoparticles are made of gold.

32.   A method of detecting nucleic acid having at least two portions comprising:

contacting the nucleic acid with a substrate
20   having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to a first portion of the sequence of the nucleic acid, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the
25   substrate with the nucleic acid;

contacting the nucleic acid bound to the substrate with liposomes having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to a portion of the sequence of the nucleic
30   acid, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the liposomes with the nucleic acid;

contacting the liposomes bound to the substrate with a first type of nanoparticles having at least a
35   first type oligonucleotides attached thereto, the first

type of oligonucleotides having a hydrophobic group attached to the end not attached to the nanoparticles, the contacting taking place under conditions effective to allow attachment of the oligonucleotides on the

5   nanoparticles to the liposomes as a result of hydrophobic interactions; and

observing a detectable change.

33.   A method of detecting nucleic acid having at

10   least two portions comprising

contacting the nucleic acid with a substrate having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to a first portion of the sequence of the nucleic acid, the

15   contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the substrate with the nucleic acid;

contacting the nucleic acid bound to the substrate with liposomes having oligonucleotides attached

20   thereto, the oligonucleotides having a sequence complementary to a portion of the sequence of the nucleic acid, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the liposomes with the nucleic acid;

25   contacting the liposomes bound to the substrate with a first type of nanoparticles having at least a first type oligonucleotides attached thereto, the first type of oligonucleotides having a hydrophobic group attached to the end not attached to the nanoparticles,

30   the contacting taking place under conditions effective to allow attachment of the oligonucleotides on the nanoparticles to the liposomes as a result of hydrophobic interactions;

contacting the first type of nanoparticles
bound to the liposomes with a second type of
nanoparticles having oligonucleotides attached thereto,
the first type of nanoparticles having a
5   second type of oligonucleotides attached thereto which
have a sequence complementary to at least a portion of
the sequence of the oligonucleotides on the second type
of nanoparticles,
the oligonucleotides on the second type of
10   nanoparticles having a sequence complementary to at least
a portion of the sequence of the second type of
oligonucleotides on the first type of nanoparticles,
the contacting taking place under
conditions effective to allow hybridization of the
15   oligonucleotides on the first and second types of
nanoparticles; and
observing a detectable change.

34. A method of detecting a nucleic acid having at
20   least two portions comprising:
providing nanoparticles having oligonucleotides
attached thereto;
providing one or more types of binding
oligonucleotides, each of the binding oligonucleotides
25   having two portions, the sequence of one portion being
complementary to the sequence of one of the portions of
the nucleic acid and the sequence of the other portion
being complementary to the sequence of the
oligonucleotides on the nanoparticles;
30   contacting the nanoparticles and the binding
oligonucleotides under conditions effective to allow
hybridization of the oligonucleotides on the
nanoparticles with the binding oligonucleotides;
contacting the nucleic acid and the binding
35   oligonucleotides under conditions effective to allow

hybridization of the binding oligonucleotides with the nucleic acid; and

   observing a detectable change.

5   35. The method of Claim 34 wherein the nanoparticles are contacted with the binding oligonucleotides prior to being contacted with the nucleic acid.

10   36. A method of detecting nucleic acid having at least two portions comprising:

   contacting the nucleic acid with at least two types of particles having oligonucleotides attached thereto,

   the oligonucleotides on the first type of
15 particles having a sequence complementary to a first portion of the sequence of the nucleic acid and being labeled with an energy donor,

   the oligonucleotides on the second type of particles having a sequence complementary to a second portion of
20 the sequence of the nucleic acid and being labeled with an energy acceptor,

   the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the particles with the nucleic acid; and
25   observing a detectable change brought about by hybridization of the oligonucleotides on the particles with the nucleic acid.

      37. The method of Claim 36 wherein the energy donor
30 and acceptor are fluorescent molecules.

      38. A kit comprising at least one container, the container holding a composition comprising at least two types of nanoparticles having oligonucleotides attached
35 thereto, the oligonucleotides on the first type of

nanoparticles having a sequence complementary to the sequence of a first portion of a nucleic acid, the oligonucleotides on the second type of nanoparticles having a sequence complementary to the sequence of a

5 second portion of the nucleic acid.

39. The kit of Claim 38 wherein the composition in the container further comprises a filler oligonucleotide having a sequence complementary to a third portion of the

10 nucleic acid, the third portion being located between the first and second portions.

40. The kit of Claim 38 wherein the nanoparticles are made of gold.

15

41. The kit of Claim 38 further comprising a solid surface.

42. A kit comprising at least two containers,

20 the first container holding nanoparticles having oligonucleotides attached thereto which have a sequence complementary to the sequence of a first portion of a nucleic acid, and

the second container holding nanoparticles

25 having oligonucleotides attached thereto which have a sequence complementary to the sequence of a second portion of the nucleic acid.

43. The kit of Claim 42 comprising a third

30 container holding oligonucleotides having a sequence complementary to a third portion of the nucleic acid, the third portion being located between the first and second portions.

44. The kit of Claim 42 wherein the nanoparticles are made of gold.

45. The kit of Claim 42 further comprising a solid
5 surface.

46. A kit comprising at least two containers,
       the first container holding nanoparticles having oligonucleotides attached thereto which have a
10 sequence complementary to the sequence of a first portion of a binding oligonucleotide, and
       the second container holding a binding oligonucleotide which has a sequence comprising at least two portions, the first portion being complementary to
15 the sequence of the oligonucleotides on the nanoparticles and the second portion being complementary to the sequence of a portion of a nucleic acid.

47. The kit of Claim 46 which comprises additional
20 containers, each holding an additional binding oligonucleotide, each additional binding oligonucleotide having a sequence comprising at least two portions, the first portion being complementary to the sequence of the oligonucleotides on the nanoparticles and the second
25 portion being complementary to the sequence of another portion of the nucleic acid.

48. The kit of Claim 46 wherein the nanoparticles are made of gold.
30

49. The kit of Claim 46 further comprising a solid surface.

50. A kit comprising:

111

      a container holding one type of nanoparticles
having oligonucleotides attached thereto and one or more
types of binding oligonucleotides, each of the types of
binding oligonucleotides having a sequence comprising at
5   least two portions, the first portion being complementary
  to the sequence of the oligonucleotides on the
nanoparticles, whereby the binding oligonucleotides are
hybridized to the oligonucleotides on the nanoparticles,
and the second portion being complementary to the
10   sequence of one or more portions of a nucleic acid.

      50.   A kit comprising at least one container, the
container holding metallic or semiconductor nanoparticles
having oligonucleotides attached thereto, the
15   oligonucleotides having a sequence complementary to a
portion of a nucleic acid and having fluorescent
molecules attached to the ends of the oligonucleotides
not attached to the nanoparticles.

20   51.   A kit comprising:
      a substrate, the substrate having attached
thereto nanoparticles, the nanoparticles having
oligonucleotides attached thereto which have a sequence
complementary to the sequence of a first portion of a
25   nucleic acid; and
      a first container holding nanoparticles having
oligonucleotides attached thereto which have a sequence
complementary to the sequence of a second portion of the
nucleic acid. ,
30

      52.   The kit of Claim 51 further comprising:
      a second container holding a binding
oligonucleotide having a selected sequence having at
least two portions, the first portion being complementary
35   to at least a portion of the sequence of the

oligonucleotides on the nanoparticles in the first container; and

a third container holding nanoparticles having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to the sequence of a second portion of the binding oligonucleotide.

53. A kit comprising at least three containers:
the first container holding nanoparticles;
the second container holding a first oligonucleotide having a sequence complementary to the sequence of a first portion of a nucleic acid; and
the third container holding a second oligonucleotide having a sequence complementary to the sequence of a second portion of the nucleic acid.

54. The kit of Claim 53 further comprising a fourth container holding a third oligonucleotide having a sequence complementary to the sequence of a third portion of the nucleic acid, the third portion being located between the first and second portions.

55. The kit of Claim 53 further comprising a substrate.

56. The kit of Claim 55 further comprising:
a fourth container holding a binding oligonucleotide having a selected sequence having at least two portions, the first portion being complementary to at least a portion of the sequence of the second oligonucleotide; and
a fifth container holding an oligonucleotide having a sequence complementary to the sequence of a second portion of the binding oligonucleotide.

113

57.   The kit of Claim 53 wherein the
oligonucleotides, nanoparticles, or both bear functional
groups for attachment of the oligonucleotides to the
nanoparticles.

5

58.   The kit of Claim 55 wherein the substrate,
nanoparticles, or both bear functional groups for
attachment of the nanoparticles to the substrate.

10   59.   The kit of Claim 55 wherein the substrate has
nanoparticles attached to it.

60.   The kit of Claim 53 wherein the nanoparticles
are made of gold.

15

61.   A kit comprising:
a substrate having oligonucleotides attached
thereto which have a sequence complementary to the
sequence of a first portion of a nucleic acid;
20   a first container holding nanoparticles having
oligonucleotides attached thereto, some of which have a
sequence complementary to the sequence of a second
portion of the nucleic acid; and
a second container holding nanoparticles having
25 oligonucleotides attached thereto which have a sequence
complementary to at least a portion of the sequence of
the oligonucleotides attached to the nanoparticles in the
first container.

30   62.   A kit comprising:
a substrate;
a first container holding nanoparticles;
a second container holding a first
oligonucleotide having a sequence complementary to the
35 sequence of a first portion of a nucleic acid;

a third container holding a second oligonucleotide having a sequence complementary to the sequence of a second portion of the nucleic acid; and

a fourth container holding a third
5   oligonucleotide having a sequence complementary to at least a portion of the sequence of the second oligonucleotide.

63.   The kit of Claim 62 wherein the
10   oligonucleotides, nanoparticles, substrate or all bear functional groups for attachment of the oligonucleotides to the nanoparticles or for attachment of the oligonucleotides to the substrate.

15   64.   The kit of Claim 62 wherein the nanoparticles are made of gold.

65.   A kit comprising:

a substrate having oligonucleotides attached
20   thereto which have a sequence complementary to the sequence of a first portion of a nucleic acid;

a first container holding liposomes having oligonucleotides attached thereto which have a sequence complementary to the sequence of a second portion of the
25   nucleic acid; and

a second container holding nanoparticles having at least a first type of oligonucleotides attached thereto, the first type of oligonucleotides having a hydrophobic group attached to the end not attached to the
30   nanoparticles.

66.   The kit of Claim 65 wherein:

the nanoparticles in the second container have a second type of oligonucleotides attached thereto, the
35   second type of oligonucleotides having a sequence

complementary to the sequence of the oligonucleotides on a second type of nanoparticles;

and the kit further comprises:

a third container holding a second type of
5   nanoparticles having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to at least a portion of the sequence of the second type of olignucleotides on the first type of nanoparticles.

10   67.   A kit comprising at least two containers, the first container holding particles having oligonucleotides attached thereto which have a sequence complementary to the sequence of a first portion of a nucleic acid, the oligonucleotides being labeled with an
15   energy donor on the ends not attached to the particles, the second container holding particles having oligonucleotides attached thereto which have a sequence complementary to the sequence of a second portion of a nucleic acid, the oligonucleotides being labeled with an
20   energy acceptor on the ends not attached to the particles.

        68.   The kit of Claim 67 wherein the energy donor and acceptor are fluorescent molecules.
25

        69.   A kit comprising at least one container, the container holding a first type of particles having oligonucleotides attached thereto which have a sequence complementary to the sequence of a first portion of a
30   nucleic acid, the oligonucleotides being labeled with an energy donor on the ends not attached to the particles, and a second type of particles having oligonucleotides attached thereto which have a sequence complementary to the sequence of a second portion of a nucleic acid, the

oligonucleotides being labeled with an energy acceptor on
the ends not attached to the particles.

    70.   The kit of Claim 69 wherein the energy donor
5   and acceptor are fluorescent molecules.

    71.   A substrate having nanoparticles attached
thereto.

10   72.   The substrate of Claim 71 wherein the
nanoparticles have oligonucleotides attached thereto
which have a sequence complementary to the sequence of a
first portion of a nucleic acid.

15   73.   A metallic or semiconductor nanoparticle having
oligonucleotides attached thereto, the oligonucleotides
being labeled with fluorescent molecules at the ends not
attached to the nanoparticle.

20   74.   A method of nanofabrication comprising
    providing at least one type of linking
oligonucleotide having a selected sequence, the sequence
of each type of linking oligonucleotide having at least
two portions;
25   providing one or more types of nanoparticles
having oligonucleotides attached thereto, the
oligonucleotides on each of the types of nanoparticles
having a sequence complementary to the sequence of a
portion of a linking oligonucleotide; and
30   contacting the linking oligonucleotides and
nanoparticles under conditions effective to allow
hybridization of the oligonucleotides on the
nanoparticles to the linking oligonucleotides so that a
desired nanomaterial or nanostructure is formed wherein

the nanoparticles are held together by oligonucleotide connectors.

75.   The method of Claim 74 wherein the
5   nanoparticles are made of gold.

76.   The method of Claim 74 wherein at least two types of nanoparticles having oligonucleotides attached thereto are provided, the oligonucleotides on the first
10   type of nanoparticles having a sequence complementary to a first portion of the sequence of a linking oligonucleotide, and the oligonucleotides on the second type of nanoparticles having a sequence complementary to a second portion of the sequence of the linking
15   oligonucleotide.

77.   The method of Claim 76 wherein the nanoparticles are made of gold.

20   78.   A method of nanofabrication comprising:
       providing at least two types of nanoparticles having oligonucleotides attached thereto,
       the oligonucleotides on the first type of nanoparticles having a sequence complementary to that of
25   the oligonucleotides on the second of the nanoparticles;
       the oligonucleotides on the second type of nanoparticles having a sequence complementary to that of the oligonucleotides on the first type of nanoparticles; and
30   contacting the first and second types of nanoparticles under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles to each other so that a desired nanomaterial or nanostructure is formed.
35

79. The method of Claim 78 wherein the nanoparticles are made of gold.

80. Nanomaterials or nanostructures composed of nanoparticles having oligonucleotides attached thereto, the nanoparticles being held together by oligonucleotide connectors.

81. The nanomaterials or nanostructures of Claim 80 wherein at least some of the oligonucleotide connectors are triple-stranded.

82. The nanomaterials or nanostructures of Claim 80 wherein the nanoparticles are made of gold.

83. A composition comprising at least two types of nanoparticles having oligonucleotides attached thereto, the oligonucleotides on the first type of nanoparticles having a sequence complementary to the sequence of a first portion of a nucleic acid or a linking oligonucleotide, the oligonucleotides on the second type of nanoparticles having a sequence complementary to the sequence of a second portion of the nucleic acid or linking oligonucleotide.

84. The composition of Claim 83 wherein the nanoparticles are made of gold.

85. An assembly of containers comprising:
    a first container holding nanoparticles having oligonucleotides attached thereto, and
    a second container holding nanoparticles having oligonucleotides attached thereto,
    the oligonucleotides attached to the nanoparticles in the first container having a sequence

complementary to that of the oligonucleotides attached to the nanoparticles in the second container,

the oligonucleotides attached to the nanoparticles in the second container having a sequence
5   complementary to that of the oligonucleotides attached to the nanoparticles in the second container.

86.   The assembly of Claim 85 wherein the nanoparticles are made of gold.
10

87.   A nanoparticle having a plurality of different oligonucleotides attached thereto.

88.   A method of separating a selected nucleic acid
15   having at least two portions from other nucleic acids, the method comprising:

providing one or more types of nanoparticles having oligonucleotides attached thereto, the oligonucleotides on each of the types of nanoparticles
20   having a sequence complementary to the sequence of one of the portions of the selected nucleic acid; and

contacting the nucleic acids and nanoparticles under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with the selected
25   nucleic acid so that the nanoparticles hybridized to the selected nucleic acid aggregate and precipitate.
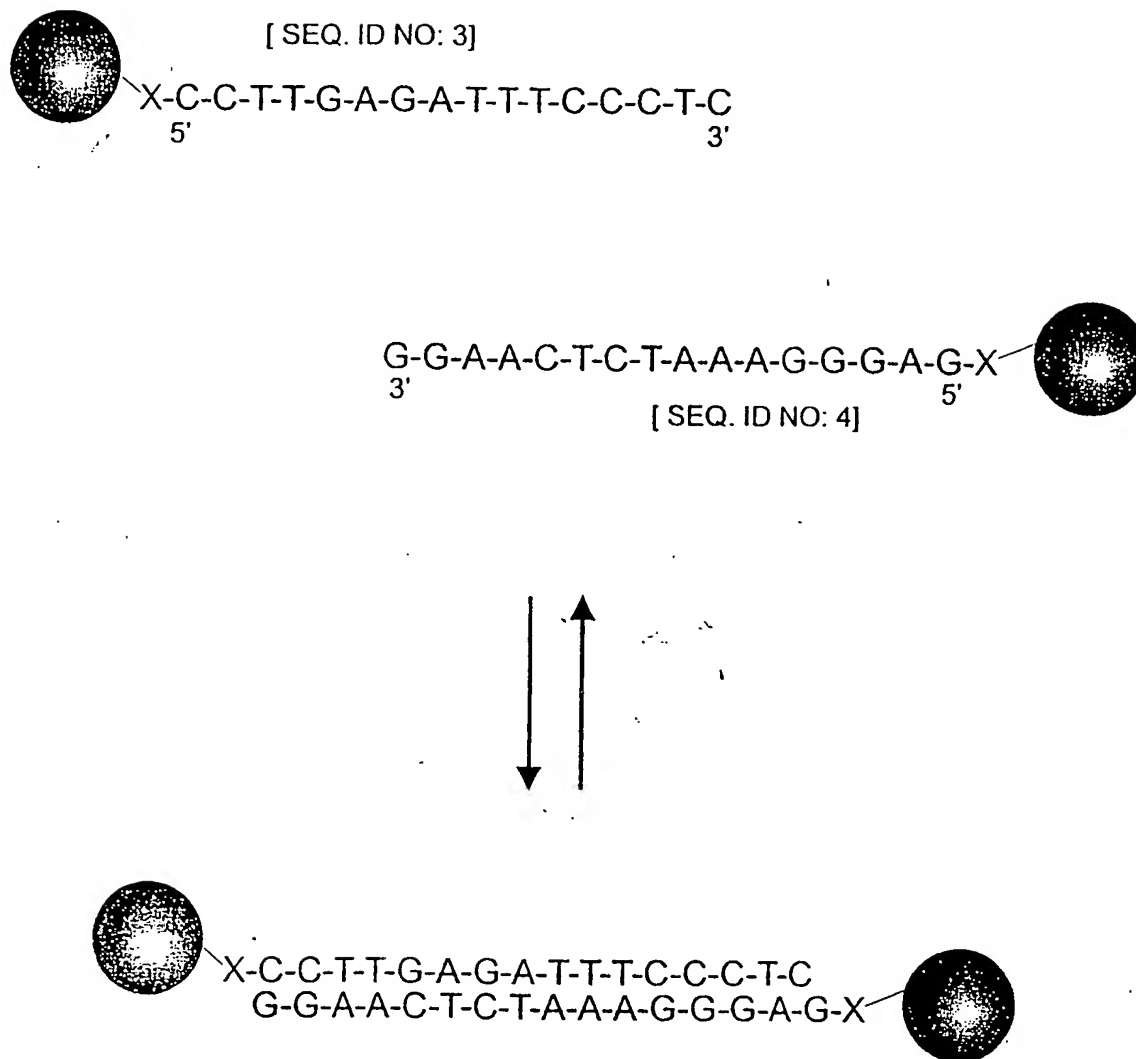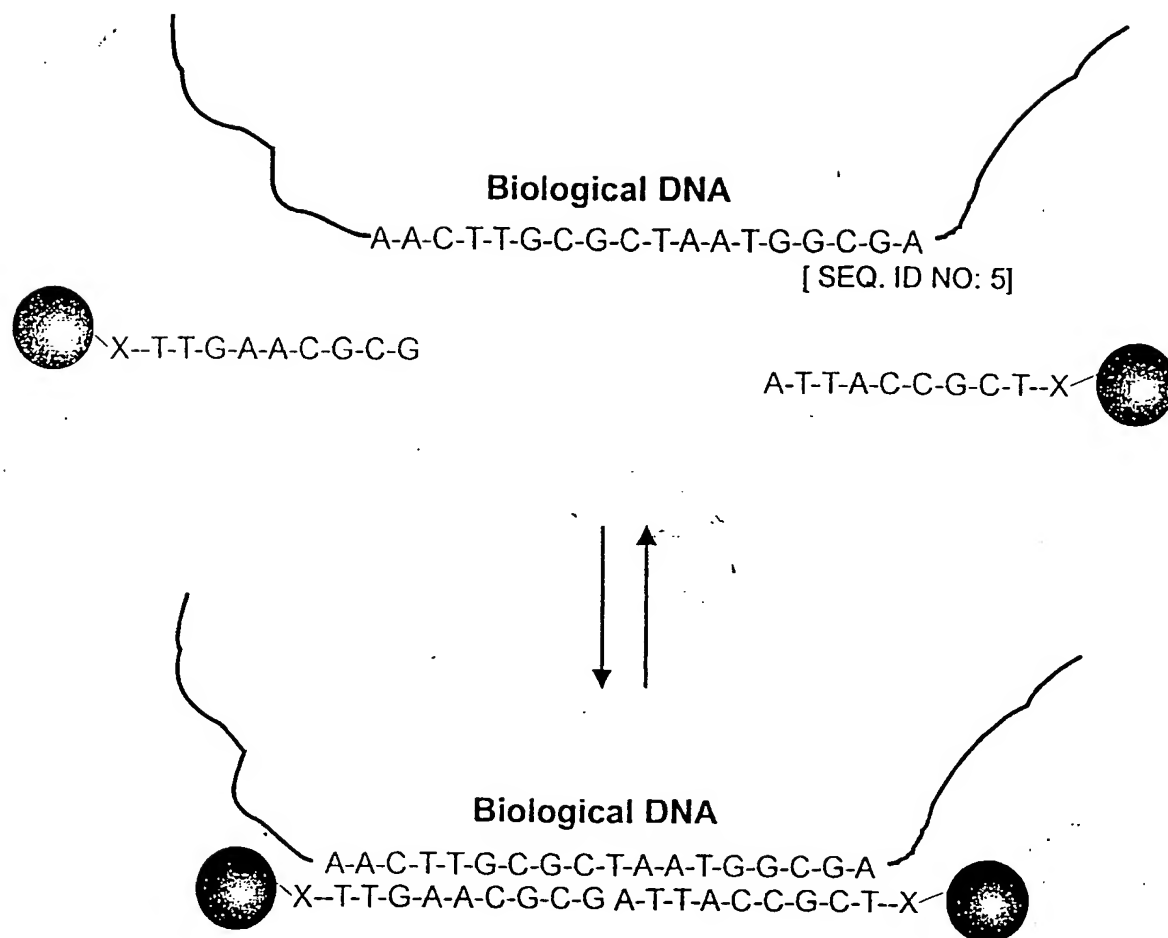
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[SEQ. ID NO: 3]

X-C-C-T-T-G-A-G-A-T-T-T-C-C-C-T-C
　5'　　　　　　　　　　　　　　　　　3'

G-G-A-A-C-T-C-T-A-A-A-G-G-G-A-G-X
3'　　　　　　　　　　　　　　　　5'
　　　　[SEQ. ID NO: 4]

X-C-C-T-T-G-A-G-A-T-T-T-C-C-C-T-C
G-G-A-A-C-T-C-T-A-A-A-G-G-G-A-G-X

Figure 1

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Figure 2

Figure 3

Linking oligonucleotide

Colloids

Aggregate

3'
A-T-G-G-C-A-A-C-T-A-T-A-C-G-C-G-C-T-A-G
A-T-A-T-G-C-G-C-G-A-T-C-T-C-A-G-C-A-A-A
5'

[SEQ. ID NO:1]

5'
A-T-G-G-C-A-A-C-T-A-T-A-C-G-C-G-C-T-A-G

[SEQ. ID NO: 2]

A-G-T-C-G-T-T-T-X
3'

X-T-A-C-C-G-T-T-G
3'
5'

Mix below Tm

Heat

Stand below Tm

Heat

A-T-G-G-C-A-A-C-T-A-T-A-C-G-C-G-C-T-A-G A-G-T-C-G-T-T-T-X
X-T-A-C-C-G-T-T-G A-T-A-T-G-C-G-C-G-A-T-C-T-C-A-G-C-A-A-A
3'
5'
3'
5'

Precipitate (formed by further cross-linking)
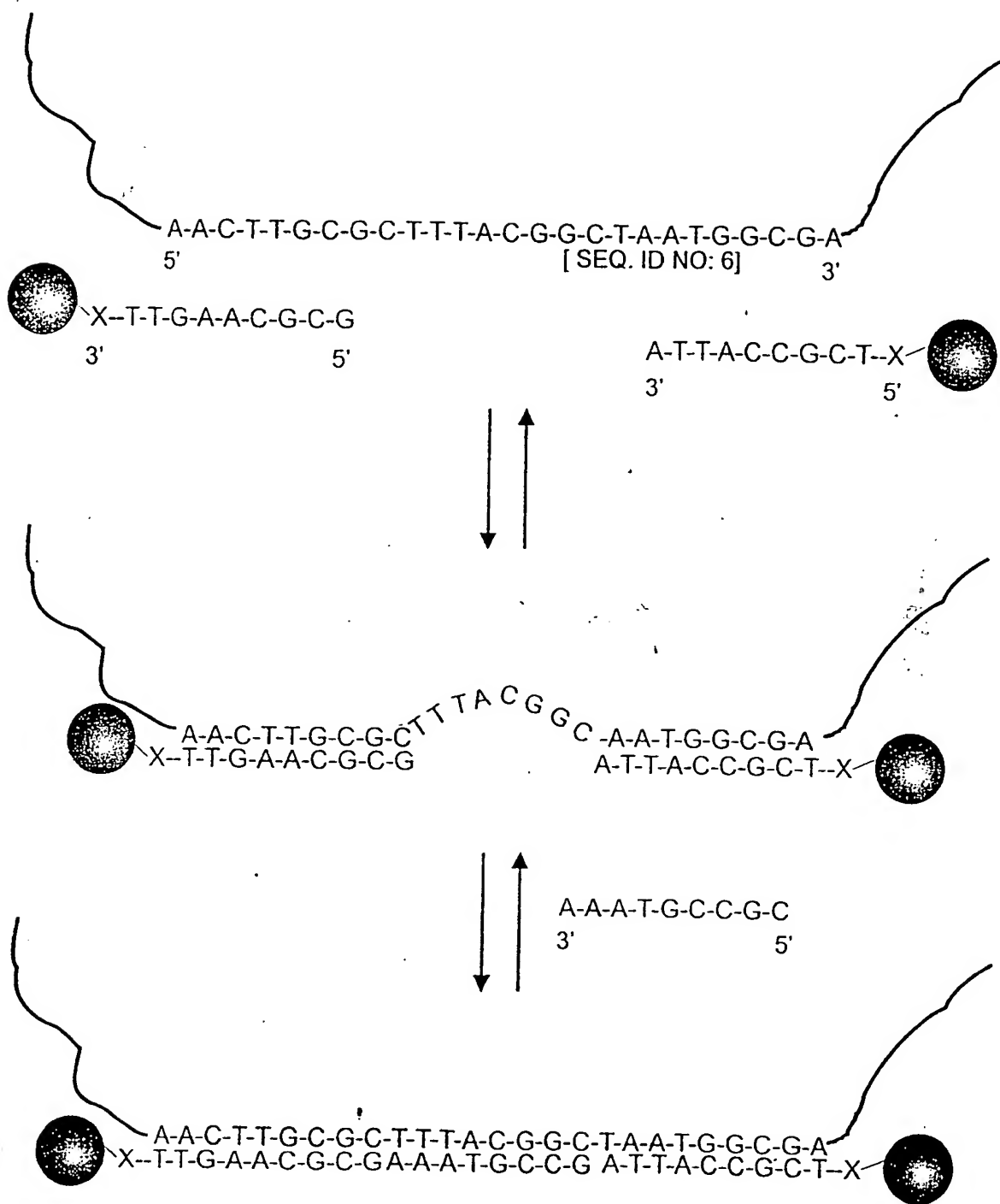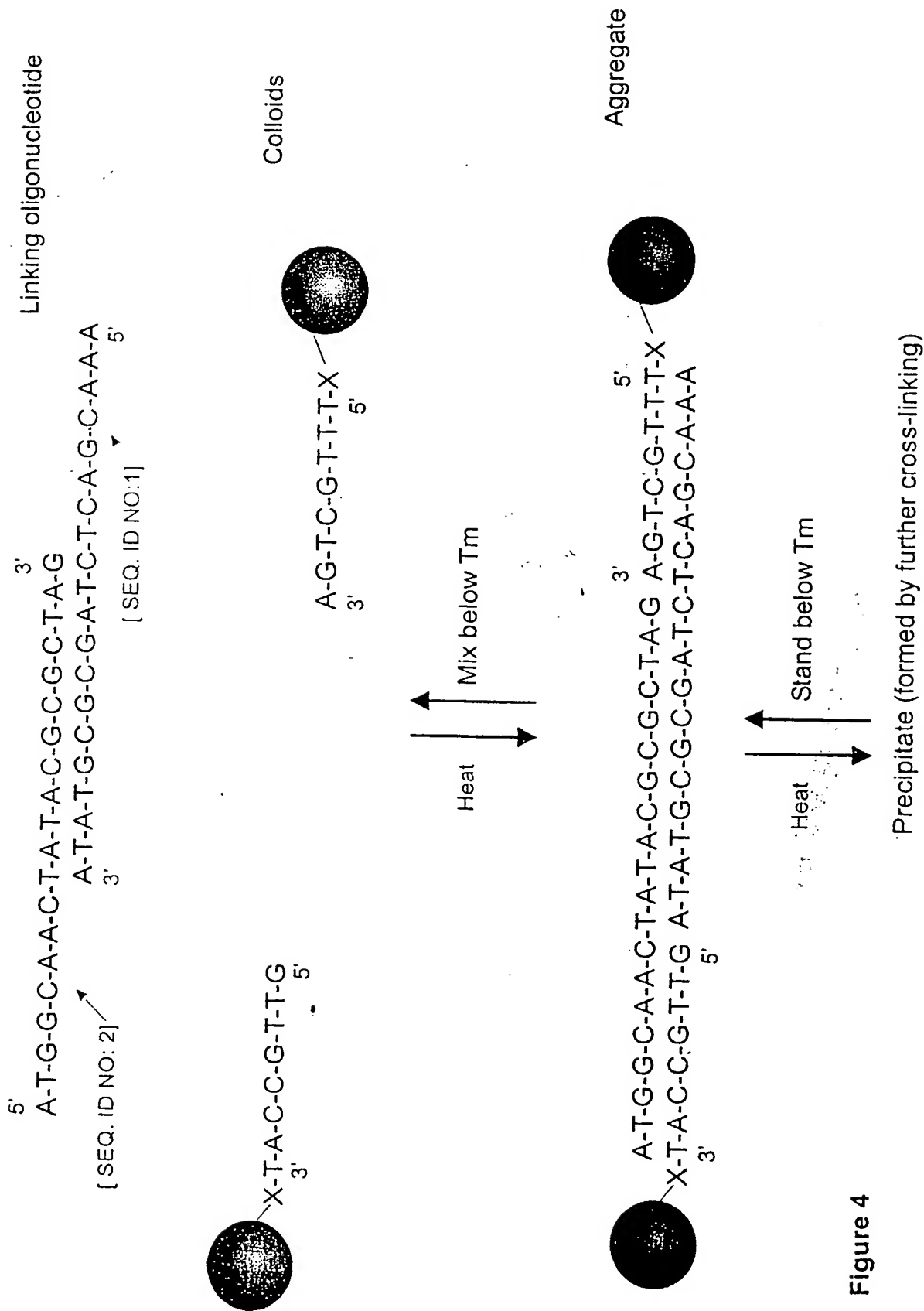
Figure 4

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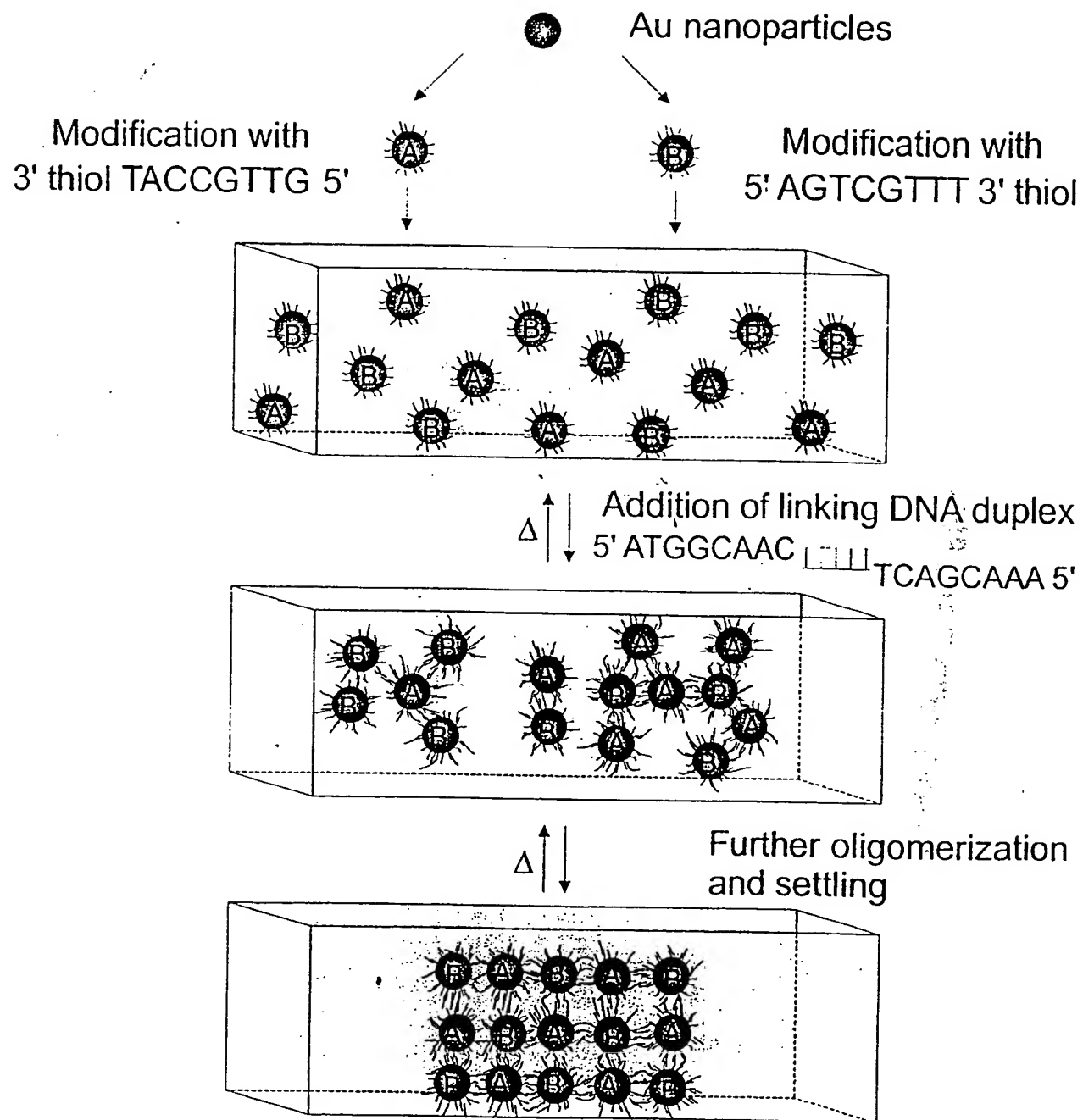
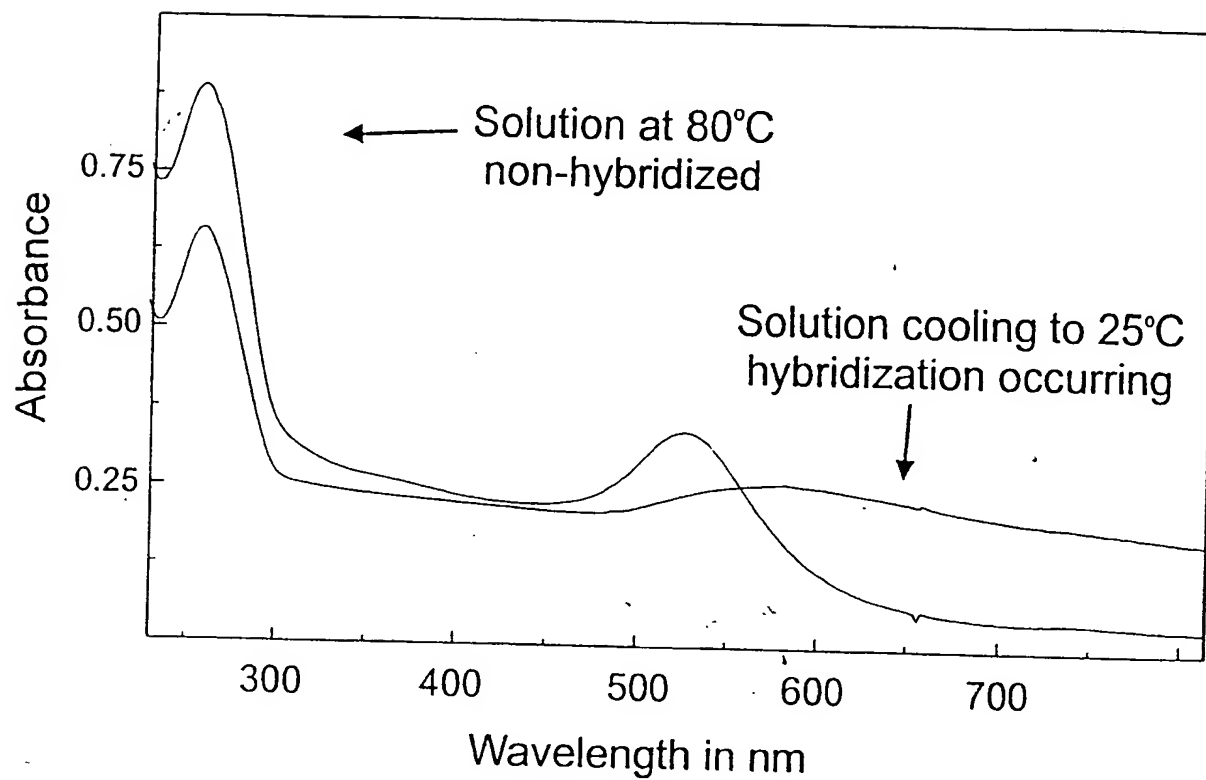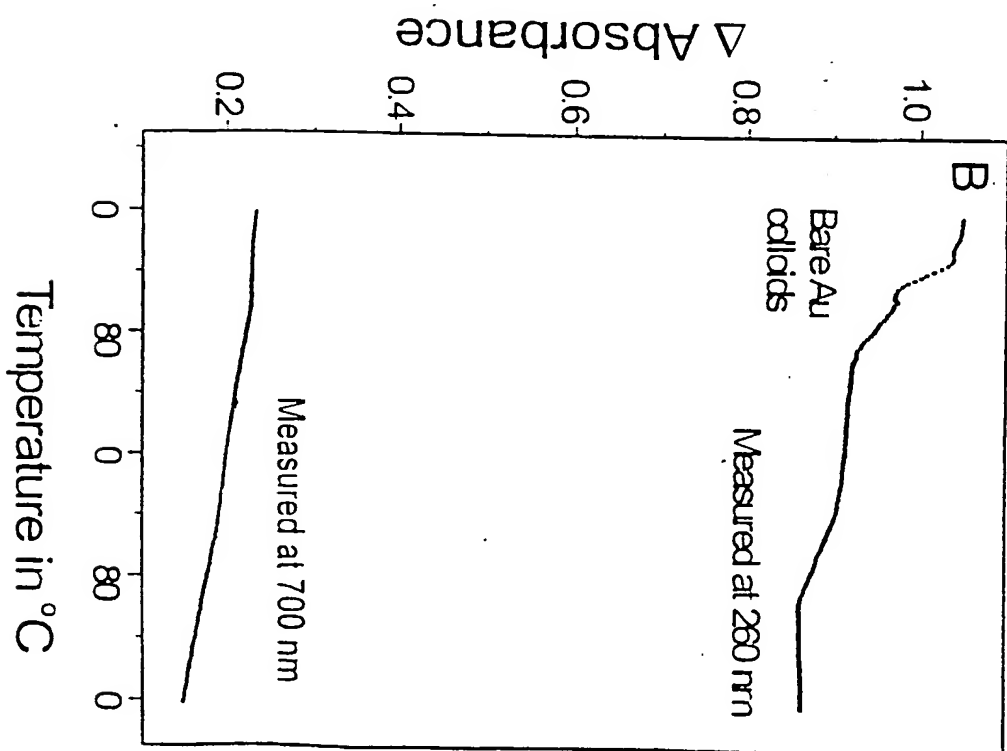Figure 5

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A   B   C
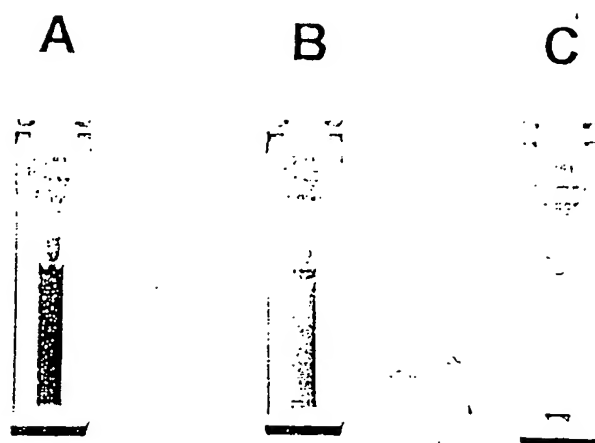
Figure 6

Figure 7

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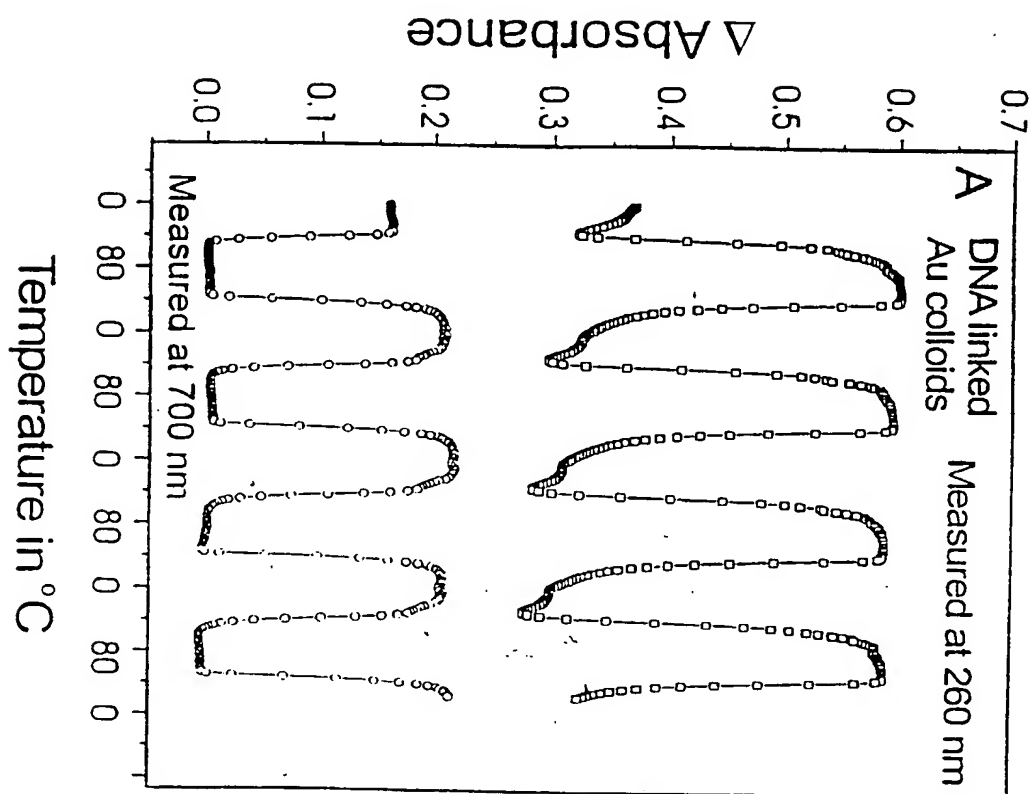
Figure 8A

Figure 8B

A

100 nm

Figure 9A

B

33 nm
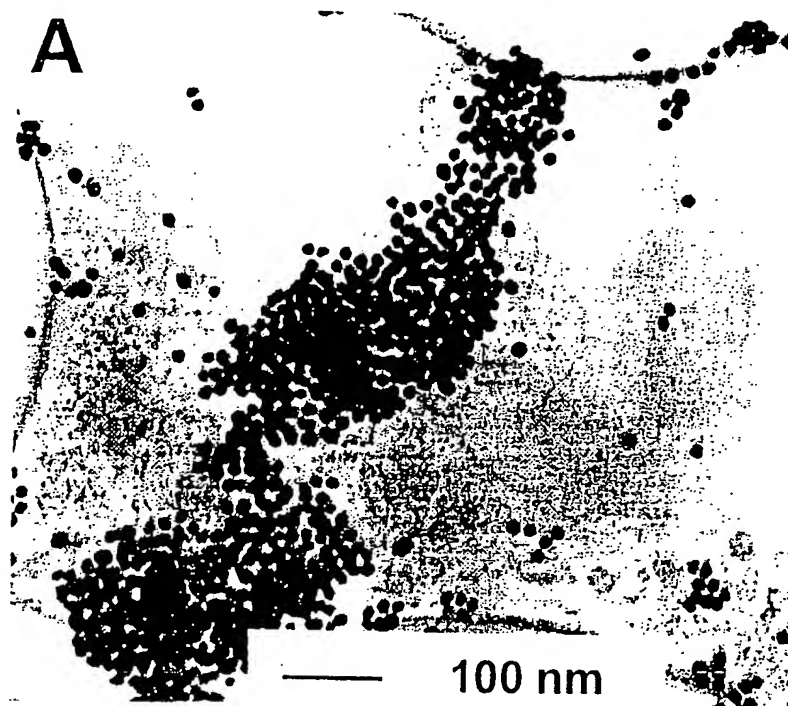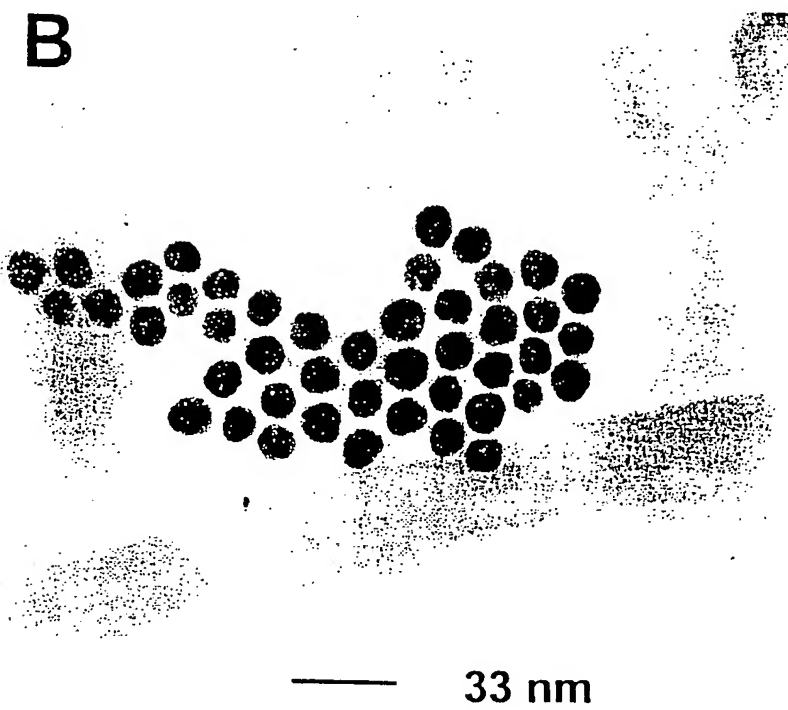
Figure 9B

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[SEQ ID NO: 7]

5'   3'
X--T-C-T-C-C-T-T-C-C-C-T-T-T-T-C
A-G-A-G-G-A-A-G-G-G-A-A-A-A-G--X
3'   5'

[SEQ ID NO: 8]

3' T-C-T-C-C-T-T-C-C-C-T-T-T-T-C 5'

[SEQ ID NO: 9]

5'   3'
X--T-C-T-C-C-T-T-C-C-C-T-T-T-T-C
A-G-A-G-G-A-A-G-G-G-A-A-A-A-G--X
3' T-C-T-C-C-T-T-C-C-C-T-T-T-T-C 5'

Figure 10

SEQ ID NO: 10

s-A-T-G-G-C-A-A-C-T-A-T-A-C-G-C-G-C-T-A-G-A-G-T-C-G-T-T-T

5'   3'

T-A-C-C-G-T-T-G-A-T-A-T-G-C-G-C-G-A-T-C-T-C-A-G-C-A-A-A–S

3'   5'

SEQ ID NO: 11

T-A-C-C-G-T-T-G-A-T-A-T-G-C-G-C-G-A-T-C-T-C-A-G-C-A-A-A–S
s-A-T-G-G-C-A-A-C-T-A-T-A-C-G-C-G-C-T-A-G-A-G-T-C-G-T-T-T

Figure 11

SEQ ID NO:14

A Complementary Target

SEQ ID NO:12

1

2

3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C   T-T-T-G-C-T-G-A-G-A-T-C-G-C-G

5' A-G-C-A-T-G-G-T-C-G-A-T-A-G-G-A-A-A-C-G-A-C-T-C-T-A-G-C-G-C

3

SEQ ID NO:13

B Probes without Target

1

2

3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C   T-T-T-G-C-T-G-A-G-A-T-C-G-C-G

C Half Complementary Target

1

2

3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C   T-T-T-G-C-T-G-A-G-A-T-C-G-C-G

5' A-G-C-A-T-G-G-T-C-G-A-T-A-G-G-A-T-G-G-C-A-A-C-T-A-T-A-C-G-C

4

SEQ ID NO:15

D Target - 6 bp

1

2

3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C   T-T-T-G-C-T-G-A-G-A-T-C-G-C-G

·5' G-T-C-G-A-T-A-G-G-A-A-A-C-G-A-C-T-C-T-A-G-C-G-C

5

SEQ ID NO:16

E One bp Mismatch

1

2

3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C   T-T-T-G-C-T-G-A-G-A-T-C-G-C-G

5' A-G-C-A-T-G-G-T-T-G-A-T-A-G-G-A-A-A-C-G-A-C-T-C-T-A-G-C-G-C

6

SEQ ID NO:17

F Two bp Mismatch

1

2

3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C-T-T-T-G-C-T-G-A-G-A-T-C-G-C-G

5' A-G-C-A-T-G-T-T-T-G-A-T-A-G-G-A-A-A-C-G-A-C-T-C-T-A-G-C-G-C

7

SEQ ID NO:18

Figure 12

Amplification Scheme for DNA Detection

A

transparent substrate

Modified DNA
chemisorbed onto
solid substrate

Analyte DNA

B'
A'

B'

transparent substrate

Analyte DNA
hybridized onto
substrate

DNA modified
colloids

B

B'

B

transparent substrate

DNA modified colloids
hybridized to bound
analyte DNA

B'

transparent substrate

Dark areas where nanoparticle
aggregates are linked to substrate
surface by analyte DNA

Figure 13A

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Alternative DNA Detection Scheme

transparent substrate

thiol terminated
modification of surface

SH SH SH SH SH SH SH SH SH
transparent substrate

gold
nanoparticles

A

transparent substrate

thiol modified
DNA adsorbed
onto particles

transparent substrate

B'
A'
analyte DNA
strand

transparent substrate

analyte DNA hybridized to
DNA modified nanoparticles

B

DNA modified
nanoparticles

B

transparent substrate

C'
B'

nanoparticle
linker strand

C'

C

transparent substrate

dark areas where
nanoparticle aggregates linked
to substrate by analyte DNA

C

DNA modified
nanoparticles

transparent substrate

Figure 13B

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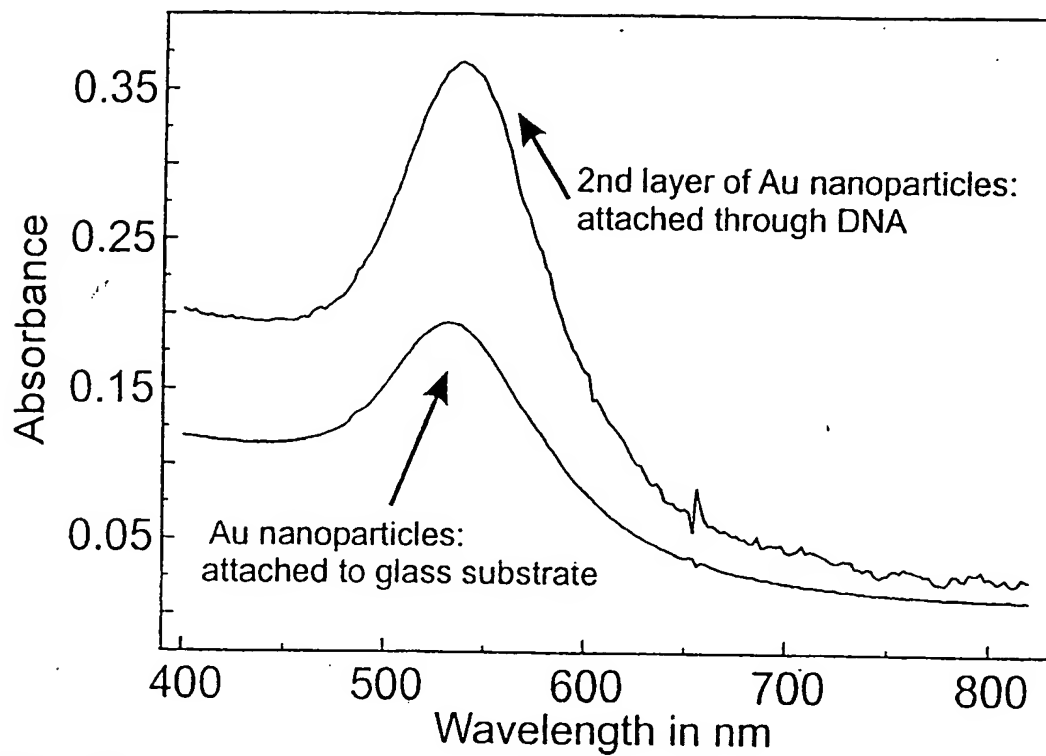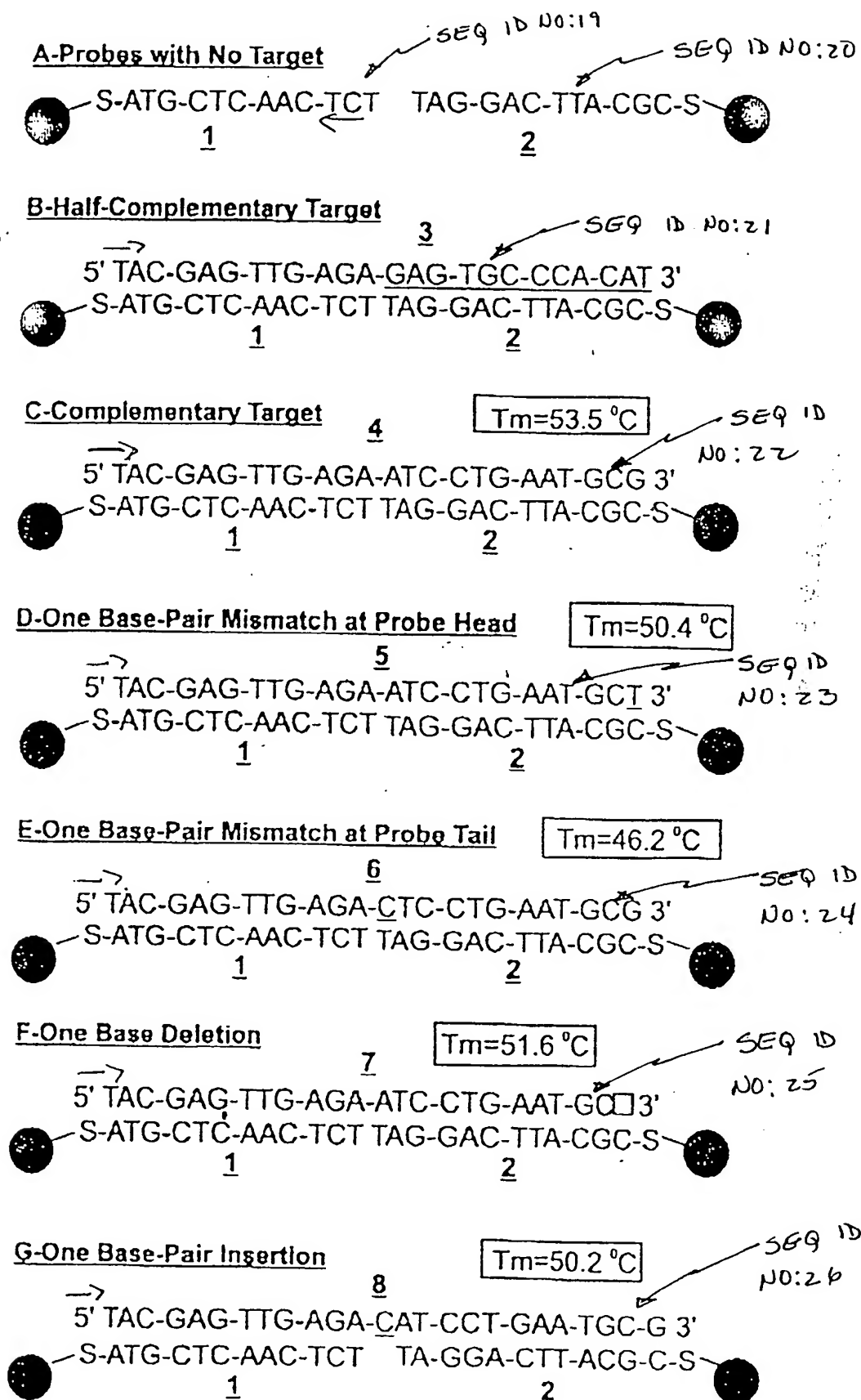
Figure 14A
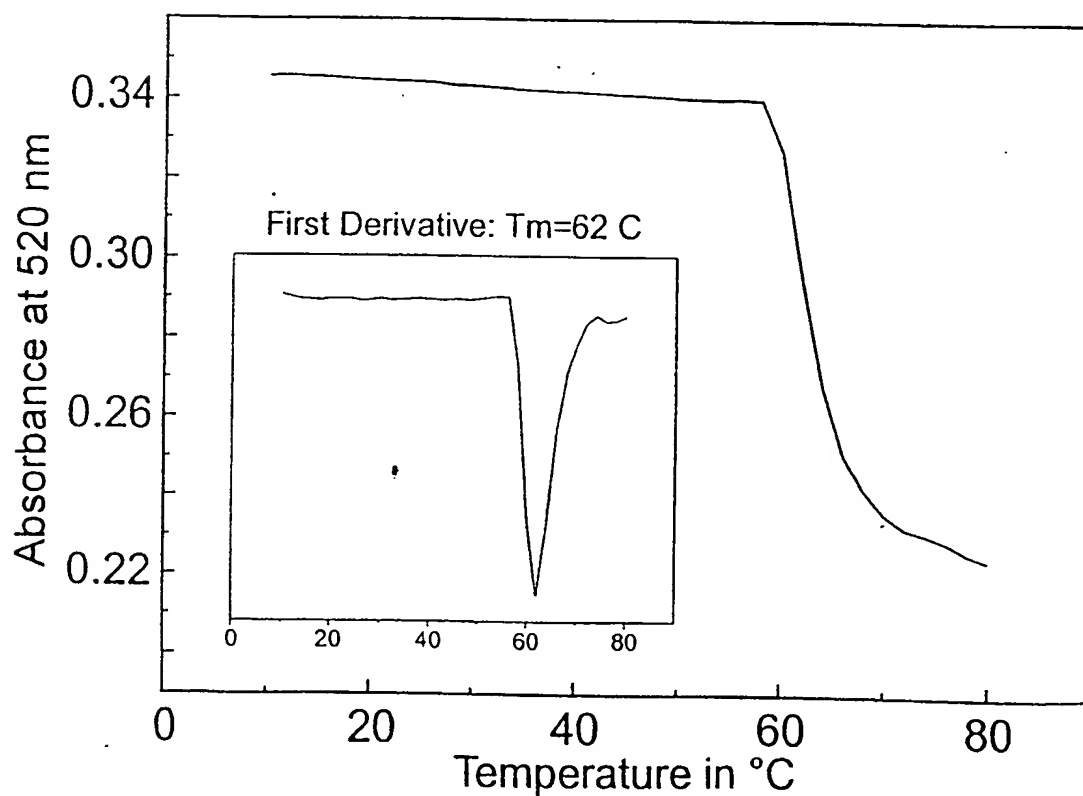
Figure 14B

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A-Probes with No Target

SEQ ID NO:19

SEQ ID NO:20

S-ATG-CTC-AAC-TCT   TAG-GAC-TTA-CGC-S

1   **2**

B-Half-Complementary Target

3

SEQ ID NO:21

→

5' TAC-GAG-TTG-AGA-GAG-TGC-CCA-CAT 3'

S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S

1   **2**

C-Complementary Target

4   Tm=53.5 °C

SEQ ID NO:22

→

5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG 3'

S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S

1   **2**

D-One Base-Pair Mismatch at Probe Head

5   Tm=50.4 °C

SEQ ID NO:23

→

5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCT 3'

S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S

1   **2**

E-One Base-Pair Mismatch at Probe Tail

6   Tm=46.2 °C

SEQ ID NO:24

→

5' TAC-GAG-TTG-AGA-CTC-CTG-AAT-GCG 3'

S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S

1   **2**

F-One Base Deletion

7   Tm=51.6 °C

SEQ ID NO:25

→

5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GC☐ 3'

S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S

1   **2**

G-One Base-Pair Insertion

8   Tm=50.2 °C

SEQ ID NO:26

→

5' TAC-GAG-TTG-AGA-CAT-CCT-GAA-TGC-G 3'

S-ATG-CTC-AAC-TCT   TA-GGA-CTT-ACG-C-S

1   **2**

Figure 15

A-24 Base Template

SEQ ID NO: 27

5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG 3'

S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S

SEQ ID NO: 28

SEQ ID NO: 19

1

2

B-48 Base Template with Complementary 24 Base Filler

SEQ ID NO: 29

5' TAC-GAG-TTG-AGA-CCG-TTA-AGA-CGA-GGC-AAT-CAT-GCA-ATC-CTG-AAT-GCG 3'

S-ATG-CTC-AAC-TCT GGC-AAT-TCT-GCT-CCG-TTA-GTA-CGT TAG-GAC-TTA-CGC-S

SEQ ID NO: 30

1

2
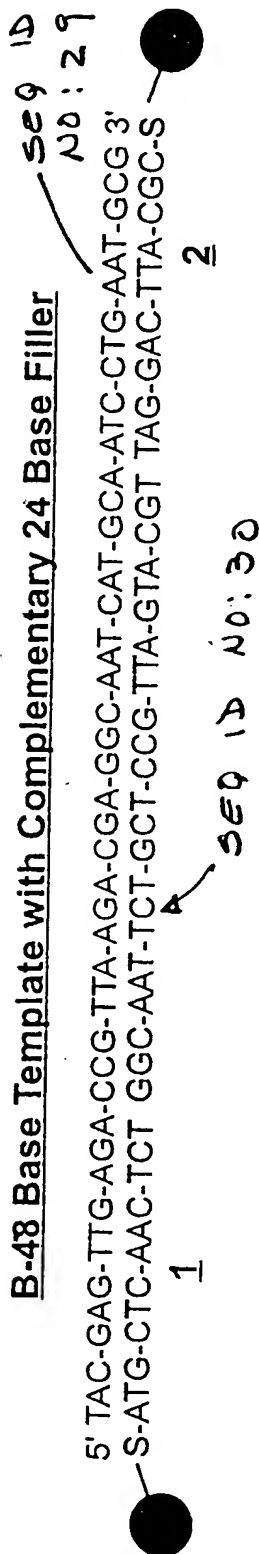
C-72 Base Template with Complementary 48 Base Filler

SEQ ID NO: 31

5' TAC-GAG-TTG-AGA-CCG-TTA-AGA-CGA-GGC-AAT-CAT-GCA-TAT-ATT-GGA-CGC-TTT-ACG-GAC-AAC-ATC-CTG-AAT-GCG 3'

S-ATG-CTC-AAC-TCT GGC-AAT-TCT-GCT-CCG-TTA-GTA-CGT-ATA-TAA-CCT-GCG-AAA-TGC-CTG-TTG TAG-GAC-TTA-CGC-S

SEQ ID NO: 32

1

2
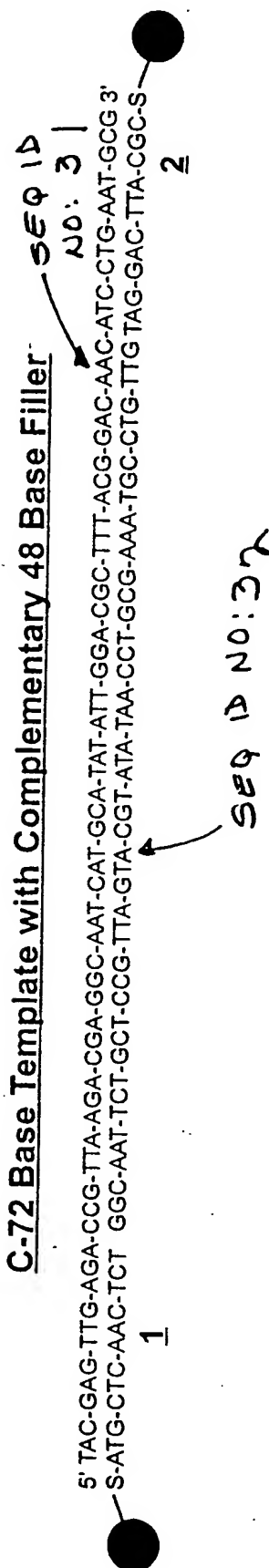
Figure 16

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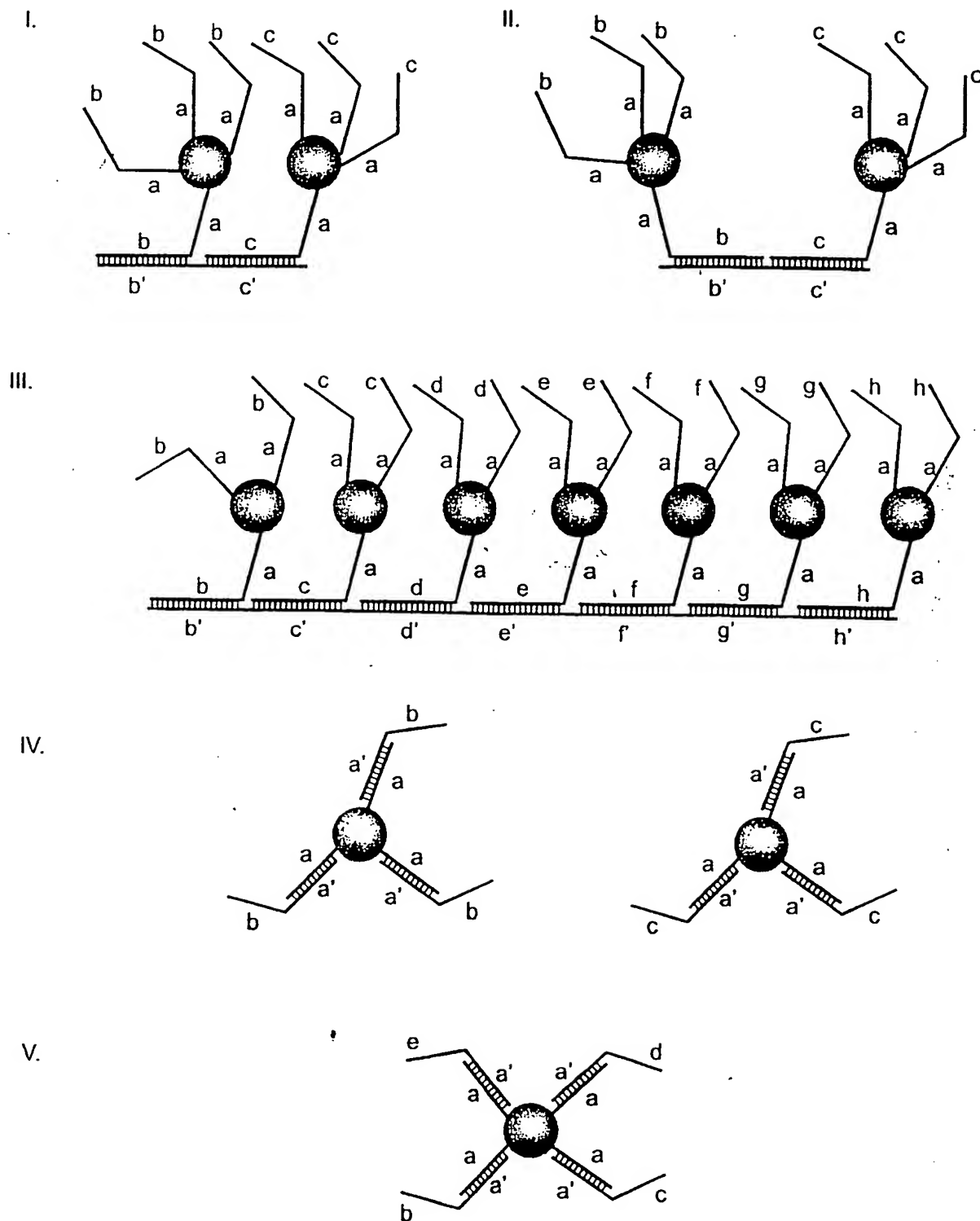
Figure 17

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Figure 18
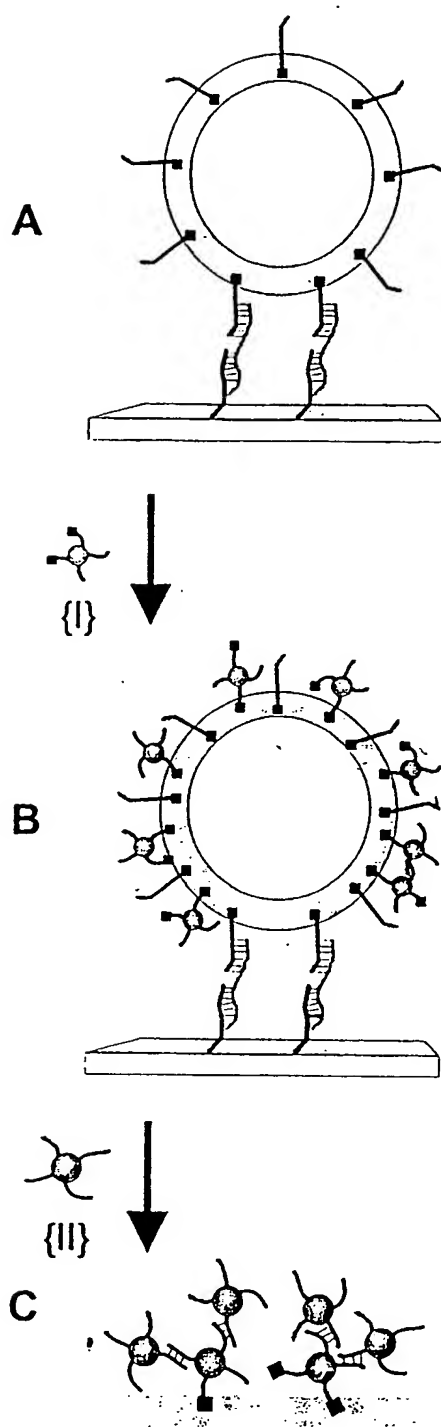
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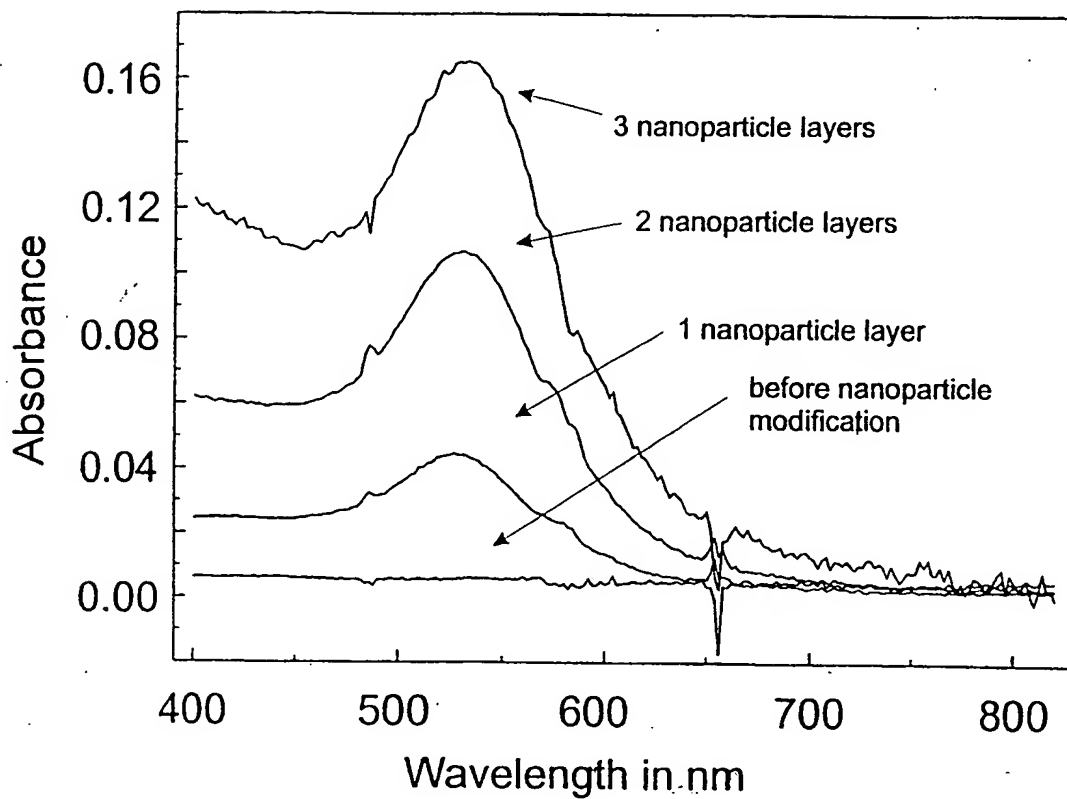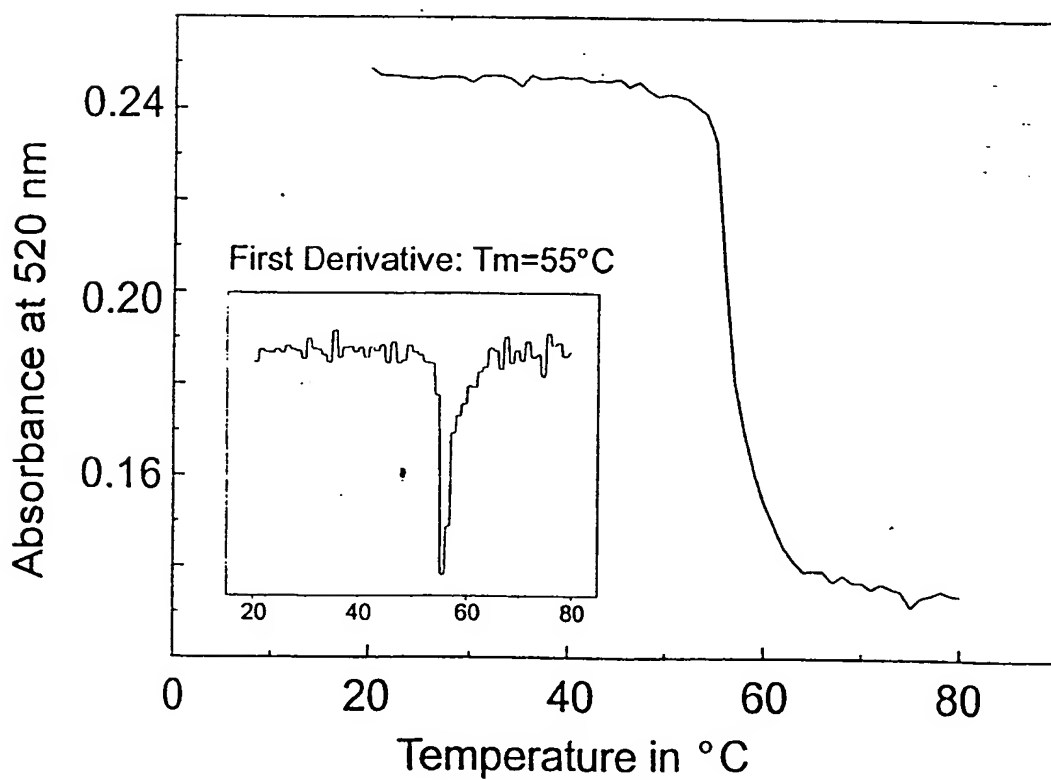
FIG. 19A

FIG. 19B

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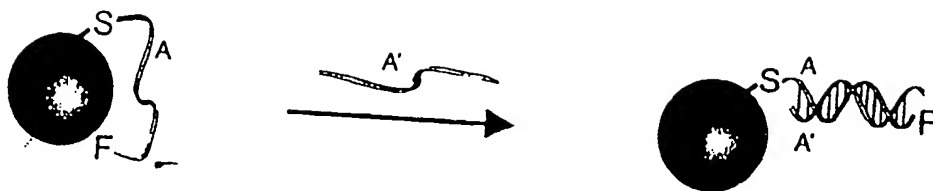
FIG. 20A.
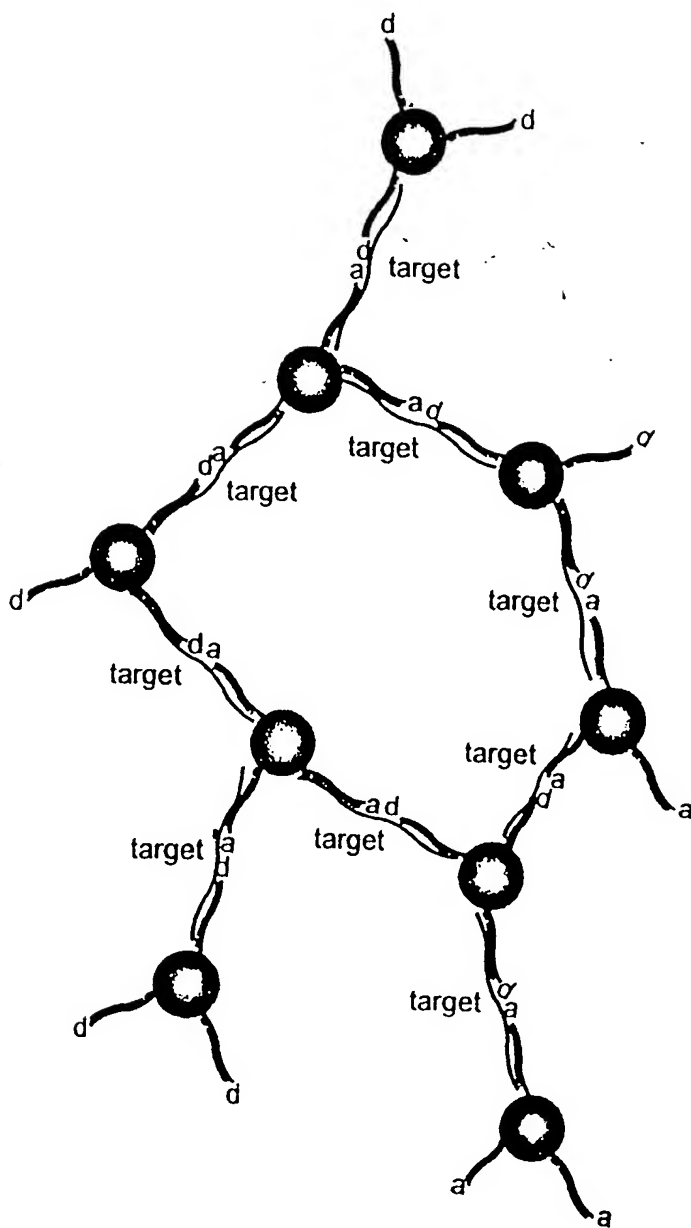
FIG. 20B

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/12783

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68.; C12P 19/34; C07H 21/02, 21/04

US CL :435/6, 91.1; 536/23.1, 24.3, 24.32, 24.33

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.1; 536/23.1, 24.3, 24.32, 24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	US 5,543,158 A (GREF et al.) 06 August 1996, see entire document.	1-88
A	US 5,460,831 A (KOSSOVSKY et al.) 24 October 1995, see abstract.	1-88
A, E	US 5,665,582 A (KAUSCH et al.) 09 September 1997, column 3, lines 7-10.	1-88

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 SEPTEMBER 1997	24.12.97

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer DIANNE REES
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, BIOSIS, BIOTECHABS, BIOBUSINESS,CABA, CASPLUS, EMBASE, CANCERLIT, AIDSLINE, JAPIO, MEDLINE, TOXLINE, TOXLIT, SCISEARCH, DISSABS, EUROPATFULL, EUROPEX, WPIDS, USPATFULL
search terms: nanoparticles, colloids or colloidal, oligonucleotides, polynucleotides, DNA, nucleic acids, hybridization, hybridisation, probes, primers, FET, energy transfer, donor acceptor, quenchers, quenching, gold, liposomes